

ANNALS OF BOTANY

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Turgor Pressure and Wall Pressure

BY

D. THODAY

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THE note by Haines (1951) on the dynamics of cell expansion still leaves room for some further clarification of the issue raised by Burström's (1948) contention, referred to in a previous communication (Thoday, 1950), that in an expanding cell the turgor pressure must exceed the wall pressure.

It is true that where changes are being propagated there must be gradients. In the particular case of the expanding cell, however, it is not obvious that there must be a gradient of pressure between cell contents and cell wall. The primary difficulty is to envisage any increase of hydrostatic pressure if there is not an equal and opposite pressure to balance it.

The difficulty may be illustrated by considering the parallel case of Pfeffer's osmometer with the manometer closed. Water enters through the membrane and the pressure registered increases. From the standpoint of Haines, not only must the difference at any moment between the osmotic potential and the registered hydrostatic pressure be considered, but the hydrostatic pressure of the solution must be regarded as greater than the pressure of the air in the manometer—otherwise the air would not be compressed. If so, however, it is further necessary to envisage a gradient of pressure within the solution (additional to any hydrostatic gradient due to level) and even a gradient of compression in the air itself.

Consider as a reverse case a turgid cell in the wall of which a small puncture is made: the sap is expressed through the puncture, the effective gradient being the difference between the hydrostatic pressures of the fluid inside and outside the cell. The hydrostatic pressure within is maintained by the pressure of the distended wall, which relaxes as the hydrostatic pressure falls. If there were no distension there would be no pressure and the sap would not be expelled. To suppose a gradient from wall to contents is therefore equivalent to assuming a time-lag between the diminution of pressure of the sap and the relaxation of the wall. With a perfectly elastic membrane and an incompressible fluid the time-lag would be infinitesimal. Thus any lag would represent time needed for adjustment of cohesion forces within the substance of the wall. Similarly, with a compressible fluid, for theoretical completeness it would be necessary to allow for a gradient of pressure within the cell from the circumference towards the puncture.

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Conversely, in a cell expanding osmotically, Haines should include a gradient of hydrostatic pressure in the cell sap, as well as a difference between the hydrostatic pressure at the surface of the protoplast and the back pressure of the wall.

The main point at issue here, as Haines implies, is in regard to quantitative significance and relative importance. In bladders as small as plant cells, with walls as elastic as cellulose and contents as incompressible as water, any time-lag in the transmission of pressure would be insignificant in relation to the rate of passage of water through the cell membranes. Moreover, the moving force responsible for change of volume is the force which causes the passage of water into the cell. The pressure change is consequential. If the entering molecules do not immediately expand the cell they must compress the sap. It is difficult to imagine the resulting pressure taking an appreciable time to communicate itself to the wall, especially as the water is entering at the surface of the cell.

While, therefore, a difference may theoretically exist between the wall pressure and the hydrostatic pressure in a cell expanding osmotically, if this is the difference to which Burström refers he has grossly exaggerated it in his diagram and given undue significance to it in his treatment of the subject.

Broyer (1950), however, has drawn special attention to Burström's definition of turgor pressure as 'the difference in osmotic values of cell sap and external solution' and his view of turgor pressure as being 'of fundamentally different physical origin from wall pressure'. From this angle it appears that the difference between 'turgor pressure' (in Burström's sense) and wall pressure, in an expanding cell, may be far from infinitesimal. In fact, for complete clarity another term is needed, viz. hydrostatic pressure, H , which is synonymous with turgor pressure in the commonly accepted sense. Burström makes $T > W$. He would probably also put $T > H$. Whether with Haines he would put $H - W > \text{zero}$ is uncertain.

Burström's emphasis of the difference in kind between 'turgor pressure' as he defines it (which for a cell in water is the 'osmotic pressure' of the sap) and wall pressure does bring out a genuine difficulty in the elementary theory of turgor and osmosis: that is the putting into one equation of expressions at first sight purporting to represent forces of quite different kinds. To bring hydrostatic pressures and osmotic 'potentials' (Thoday, 1950) into relation with each other, mediation is required. Meyer (1938) finds the necessary bridge or unifying concept in 'water diffusion pressure' which is affected by both osmotic and hydrostatic conditions. Broyer (1947) generalizes in terms of free energy—a more abstract idea, less easy for the non-physicist, not fully abreast with modern thermodynamics, to grasp, but related to the idea of potentials (Thoday, 1950) since a difference of potential represents energy available under appropriate conditions for the production of change and the performance of work.

The difficulty is, however, more apparent than real, for the expressions for osmotic potentials are in fact hydrostatic pressures, though hypothetical ones.

In each case it is the hydrostatic pressure which would so alter the internal condition of the solution as to bring its water-diffusion potential (the escaping tendency of water in the solution—Broyer, 1947, p. 4) into equivalence with that of water, i.e. abolish the water diffusion potential deficit. Alternatively, put more simply in terms of the osmometer, it is in each case the hydrostatic pressure which would just prevent the entry of pure water into the solution through a semipermeable membrane.

Burström's proposal to change the accepted meaning of turgor pressure appears particularly inappropriate since the conception of turgor is primarily hydrostatic and mechanical in its relation to the rigidity of the plant body. Dynamic ideas enter when the development and maintenance of turgor come up for consideration. Moreover, in relation to the mechanics of the plant it is not wall pressure which is immediately applicable, but the hydrostatic pressure of the sap and the *tensions* in the wall. For this reason, as well as to avoid confusion, it seems better to retain the term turgor pressure for this hydrostatic pressure.

POSTSCRIPT. Since the above was written a paper by Levitt (1951) has come to hand. He makes explicit that turgor pressure can be envisaged as acting on the general surface of the wall, while at the same time water may be diffusing through the 'pores' in it. His proposals for reform of the symbols used for the different factors in the water relations of a cell merit careful consideration with a view to general adoption.

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The Dynamics of Cell Expansion by Turgor

BY

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With two Figures in the Text

ABSTRACT

In changes of cell volume, the mean turgor pressure throughout the cell is greater or less than the wall pressure, depending on the direction of water flow. When measured, however, indefinitely close to the wall, the turgor pressure is always exactly equal to the wall pressure, the law of the equality of action and reaction being true of dynamic as well as of static states.

IN a recent note under the above title Haines (1951) has criticized some remarks of Thoday (1950) on a paper by Burström (1948). The point at issue is whether turgor pressure is always exactly equal to wall pressure, Thoday maintaining that they are while Haines and Burström take the opposite view. Haines expresses his objection in these words: 'if the turgor pressure never exceeds the wall pressure, what makes the cell expand?' This consideration seems to lead to the conclusion that Newton's Third Law, that action and reaction are equal and opposite, is 'only applicable to, and necessarily true of, static states'. Something is clearly wrong here, for the Third Law is usually included in the category of Laws of *Motion*, but before touching on the paradox of how equal and opposite forces can be consistent with a state of motion, the present author would like to offer the following admittedly simplified analysis, which may help to clarify the actual state of affairs obtaining during changes in cell volume.

Let Fig. 1 represent a small portion of the cell wall. Imagine the latter to be provided with pores through which water moves into and out of the cell. Then selecting a small element $ABCD$ of the cell contents, clear of pores and bounded by stream-lines on its sides, we can investigate the forces acting on it as follows. Let the wall pressure (i.e. the pressure exerted by the wall on the contents) along AB be p_w , and the turgor (i.e. the hydrostatic) pressure along CD be p_t . Further, let the areas of the faces AB , CD be each A_1 , and of the sides of the element bounded by flow lines A_2 . Then the forces on the element in a direction normal to the cell wall will be (Fig. 2):

$p_w \cdot A_1$ due to the wall, and directed inwards;

$p_t \cdot A_1$ due to the turgor, and directed outwards;

$f.A_2$ due to the viscous drag of the entering or escaping fluid, f being a coefficient depending, *inter alia*, on the viscosity and rate of water flow.

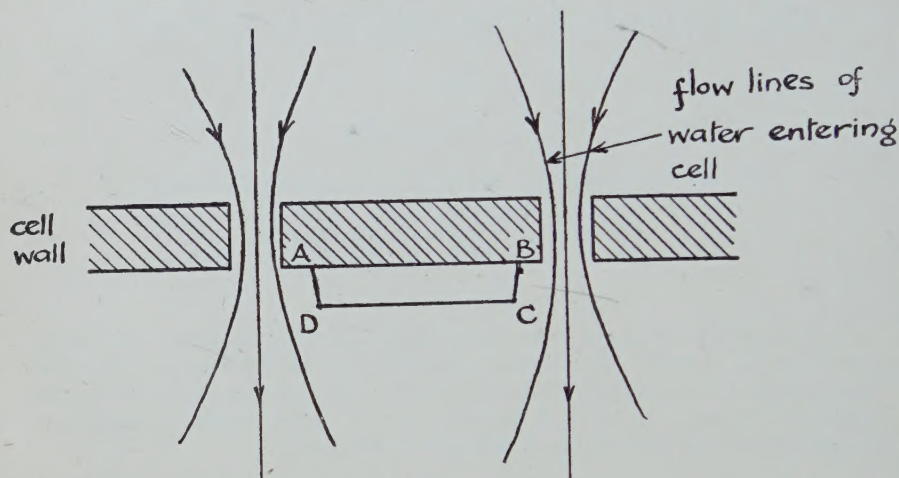


FIG. 1

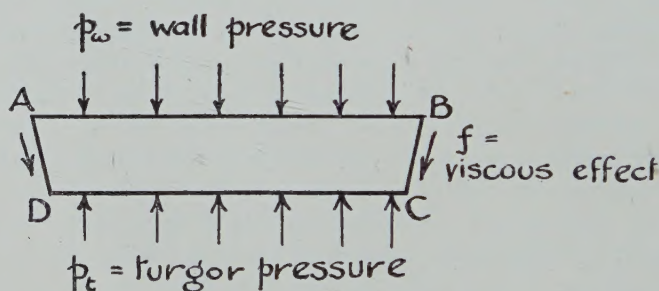


FIG. 2

The net outward force on the element will therefore be

$$p_t A_1 - p_w A_1 \pm f A_2,$$

and equating this to ma , where m is the mass of the element and a its acceleration outwards, we get for its equation of motion,

$$p_t A_1 - p_w A_1 \pm f A_2 = ma,$$

or

$$p_t - p_w = \frac{ma \pm f A_2}{A_1}. \quad (1)$$

This equation shows that in general turgor pressure and wall pressure are not equal, but differ by a term depending on both viscosity and inertia (though the latter is probably very small). Further, it indicates that when a cell is in a state of change the turgor pressure itself is not constant, but changes from

place to place—i.e. as the quantities m , a , f , and A_2 change. This naturally affects the point at issue, namely, whether turgor pressure and wall pressure are necessarily equal. There are two cases, however, for which this matter is unambiguous. In the first place, if we imagine the cell to be in perfect equilibrium the quantities a and f are both zero, and hence we have everywhere

$$p_t = p_w. \quad (2)$$

In the second place, if we make the element $ABCD$ indefinitely thin, then again (2) holds, since in this case m and A_2 both become zero. As a general statement therefore we can say that wall pressure and turgor pressure are always exactly equal and opposite *if the latter is measured indefinitely close to the former*, and this is what is meant, in the present context, by the statement that action and reaction are equal and opposite.

The above discussion has been simplified by considering pressures over an area of cell wall clear of pores. When mean pressures over larger areas (i.e. embracing pores as well) are considered a little confusion is apt to arise unless we understand clearly what we mean by 'pressure'. When a membrane separates two solutions and the whole system is in equilibrium, the resultant force on the membrane is unambiguously due to pressure. When, however, the system is not in equilibrium this is no longer the case. Liquid molecules will be passing through the membrane and exerting a frictional drag on it,¹ and the membrane will experience a force due partly to the true pressure difference on its two sides and partly to this frictional effect, which is not strictly a pressure at all. However, in our case it is probably better to define the 'wall pressure' of a cell taking in or giving out water as the total force per unit area exerted by the wall on the water, irrespective of whether it is due to 'reflection' of water molecules at its surface or to a frictional effect exerted on molecules which are traversing it. If a similar convention is adopted for the turgor pressure at the wall (which actually may vary greatly according to whether it is measured opposite a pore or not), then what has been deduced above for the simpler case will still hold for the more complex one.

It seems, therefore, to the author that the situation can be summarized in this way. When a cell is taking in water its turgor pressure is not uniform, but rises broadly speaking from the circumference inwards (and vice versa) and its *mean* turgor pressure therefore exceeds the wall pressure (see equation (1)). But the turgor pressure indefinitely close to the wall is *always* exactly equal to the wall pressure, Newton's law of action and reaction being strictly applicable. That this law is true for dynamic states is a necessary foundation for the Principle of the Conservation of Momentum. The paradox of equal and opposite forces being consistent with a state of motion disappears when we remember that action and reaction are not *two* forces, but rather two aspects of the *same* force; and that they do not both act on *one* body, but one respectively on each of the two bodies interacting. Their equivalence,

¹ This is, of course, true of equilibrium conditions also, but here the frictional forces cancel out.

therefore, does not rule out the possibility of movement, even in the presence of such frictional forces as viscosity.

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- THODAY, D., 1950: On the Water Relations of Plant Cells. *Ibid.*, xiv. 1.

Some Theoretical Considerations of Cell Water Relations

BY

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With two Figures in the Text

ABSTRACT

An analysis of the forces exerted during the osmotic changes in volume of plant cells shows that the outwardly directed turgor pressure and the inwardly directed wall pressure are numerically equal under dynamic as well as static conditions. The suggestion that the term 'potential' should replace 'pressure' in all cases except where reference is to a hydrostatic pressure, is strongly supported.

BURSTRÖM (1948) in discussing certain theoretical aspects of cell water relations proposed a new definition of turgor pressure, which assumed that the turgor pressure and the wall pressure need not be equal. Thoday (1950) pointed out the fallacy in this argument. Action and reaction being equal and opposite, the tension in the cell wall is numerically equal to the hydrostatic pressure inside the cell. More recently Haines (1951) has called Thoday's analysis into question, stating that action and reaction are only equal and opposite in a static state, and that during osmotic expansion the action (turgor pressure) must be greater than the reaction (wall pressure) or the expansion could not occur. This argument is fallacious. Thoday is undoubtedly correct in his analysis of the phenomenon of turgor changes in plant cells.

Newton's Third Law states that action and reaction are equal and opposite. This applies to all static systems. It is also tacitly applied in dynamic systems when we write their equations of motion, for we equate the actions, i.e. the forces producing accelerations, elastic deformations, &c., to the reactions, i.e. such quantities as (mass $\times$ acceleration), (deformation $\times$ modulus of elasticity). To examine the application of this law to osmotic systems it is proposed to consider at first a simplified model rather than the cell itself. The osmometer shown in Fig. 1 is closed at its lower end by a semi-permeable membrane and is fitted with a manometer and frictionless piston bearing a mass M . The downwardly directed force exerted by this piston is analogous with the inwardly directed force exerted by the cell wall. The osmometer contains solution and is immersed in water up to the level of the piston.

Static conditions

Under these conditions water is just prevented from entering the osmometer by the gravitational pull on the mass M , and the solution in the manometer will have risen to a height h which is a measure of the osmotic potential of the solution.

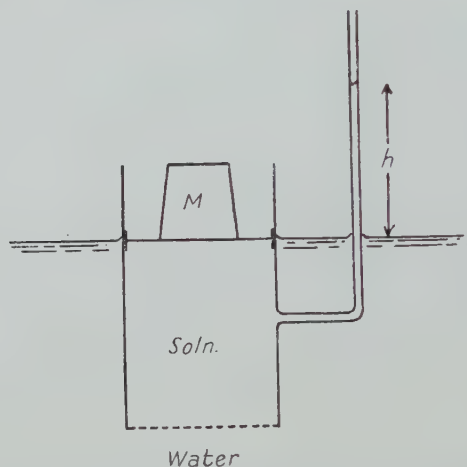


FIG. 1. Hypothetical osmometer to illustrate the forces acting during osmosis.

If the acceleration due to gravity is g , and the cross-sectional area of the piston A sq. cm., the downwardly directed force, F , exerted by the mass is given by the equation

$$F = Mg \text{ dynes.} \quad (\text{i})$$

The hydrostatic pressure in the solution $= Mg/A$ dynes per sq. cm.; hence

$$\text{Osmotic potential of solution} \equiv Mg/A = h\rho g \text{ dynes per sq. cm.,} \quad (\text{ia})$$

where ρ is the density of the solution.

Dynamic conditions

If there is a net diffusion of water into the osmometer the volume of solution in the osmometer increases and the mass is lifted. Imagine the mass to be lifted with an acceleration of a cm. per sec. per sec. The total force, F_1 , now acting on the mass is equal to the force opposing the acceleration g plus the force producing the upward acceleration. Thus:

$$F_1 = Mg + Ma = M(g + a) \text{ dynes.} \quad (\text{ii})$$

The pressure in the solution measured by the height, h_1 , of solution in the manometer will be:

$$\text{Hydrostatic pressure} = M(g + a)/A = h_1\rho g \text{ dynes per sq. cm.} \quad (\text{ii a})$$

It will be noticed that this pressure is greater than that under static conditions since there is a resultant force acting upwards equal to Ma dynes. Of course, action and reaction are still equal. The solution exerts an extra force of Ma dynes on the piston accelerating the mass upwards, and the piston exerts an extra reaction of Ma dynes downwards on the solution by virtue of the mass *being* accelerated upwards. If it did not do so the pressure in the solution would not rise to h_1 . The pressure in the solution is entirely due to the reaction on the solution of the mass on the piston. If there were no mass no pressure would develop in the solution. (That is, if the insignificant effect of the acceleration of the mass of water itself is neglected.)

When the rate of diffusion into the osmometer is slowing down, the retardation is due to a resultant force, Ma' , acting downwards, where a' cm. per sec. per sec. is the retardation. The total force, F_2 , on the mass is:

$$F_2 = M(g - a') \text{ dynes,} \quad (\text{iii})$$

and the pressure shown on the manometer, h_2 , is given by the equation:

$$h_2 \rho g = M(g - a')/A \text{ dynes per sq. cm.}$$

Thus h_2 is *less* than the pressure under static conditions although water is still passing *into* the osmometer.

If water is diffusing into the osmometer at a steady rate, a in the above equations is zero. In this case equation (ii) becomes identical with equation (i). In other words, the total forces acting on the piston when it is being lifted at a constant velocity are equal to those acting when the piston is stationary (provided that the resistance due to friction of the piston, viscosity of the solution, &c., are ignored). Also it follows that when water moves into or out of the osmometer at a constant rate the hydrostatic pressure in it is determined by the weight on the piston, and is independent of the velocity of movement (and hence independent of the osmotic potential difference across the membrane). Of course the condition when water is entering the osmometer differs from the static state in that work is being done in lifting the mass, the energy expended being stored as potential energy in the lifted mass. But the fact that work is done on the mass does not mean that the pressure in the solution exceeds the reaction of the mass. As shown above, both are equal to Mg/A dynes per sq. cm.

Applying this analysis to the plant cell it will be seen that under all conditions the hydrostatic pressure in the cell is instantaneously equal to the reaction due to the tension in the cell wall. This tension is a function of the volume of the cell, for unlike the piston and weight model the reaction of the cell wall increases as the cell increases in volume. Nevertheless when water is passing into or out of the cell at a steady rate the hydrostatic pressure in the cell at a given instant is equal to the tension in the wall as measured under static conditions at the same cell volume as that occurring at the given instant. Just as work is done in the model in lifting the mass, as the cell expands work is done in stretching the wall which is stored as potential energy in the elasticity

of the wall. But as in the model the hydrostatic pressure in the osmometer is determined by the weight on the piston, so in the cell the hydrostatic pressure is determined by the tension in the cell wall, i.e. the turgor pressure and wall pressure are necessarily equal and opposite.

The only conditions for which it might be argued from the model that turgor pressure and wall pressure are not equal are those of acceleration and retardation. When water enters the osmometer with an acceleration the pressure inside is greater than that due to the *static* reaction of the mass. However, it is precisely in this that the model and the cell are not analogous. The tension in the cell wall is a function of the volume of the cell and is independent of the rate of change in volume whether it be accelerating, retarding, or steady. Thus no extra force equivalent to Ma in equation (ii) can arise. The cell wall unlike the mass on the piston exerts a certain tension at a given volume irrespective of whether the cell is static, or is being expanded or deflated, steadily or with an acceleration or retardation. The cell differs from the model in that no resultant force can arise so that the turgor pressure must always be exactly equal to the wall pressure.

As Thoday (*loc. cit.*) has pointed out, the fallacy in Burström's interpretation of the osmotic quantities of plant cells arises out of a confusion of pressure and potential. It seems desirable to restrict the use of the term 'pressure' to mean 'hydrostatic pressure' and to replace it by the term 'potential' in all other cases, e.g. osmotic potential. The use of the term 'diffusion pressure' can equally lead to confusion as in Burström's paper, and it is suggested that the term 'diffusion potential' is more appropriate. The diffusion potential of water can be altered in two ways. It is increased or decreased by raising or lowering the hydrostatic pressure in the water. It is decreased by the presence of solutes dissolved in the water. The term 'diffusion potential' makes no implications as to the mechanism by which these factors alter the potential, but simply requires that there is a net diffusion of water from places of higher to places of lower diffusion potential.

Since only differences of diffusion potential can be measured, the diffusion potential of pure water at normal atmospheric pressure may be taken as zero. The diffusion potential of water in a system may be measured as the hydrostatic pressure above or below atmospheric pressure that must be exerted on the water to bring its diffusion potential to zero. Schofield (1949) has suggested that a convenient unit would be the potential difference between the ends of a vertical column of water 1 metre high under the influence of gravity. One atmosphere pressure would be equivalent to 10.4 metres potential. The advantage of this unit would be its distinction from units of pressure so that confusion between potential and pressure might be avoided. In this context hydrostatic pressures are only of interest in so far as they affect diffusion potentials. It is the conversion of units of pressure into units of potential which is more desirable than the usual procedure of expressing potentials in terms of atmospheres pressure.

The diffusion potential of the water in a plant cell is, then, the resultant of

(a) the decrease in diffusion potential due to the presence of solutes and (b) the increase in diffusion potential due to the hydrostatic pressure caused by the tension in the surrounding cell wall. It is this resultant diffusion potential which is called the 'suction pressure' or 'suction potential' of the cell. But the use of the word 'suction' in this connexion is objectionable since it has definite implications associated with differences in hydrostatic pressure. It is suggested that the term resultant diffusion potential or net diffusion potential of the water in the cell would be more satisfactory.

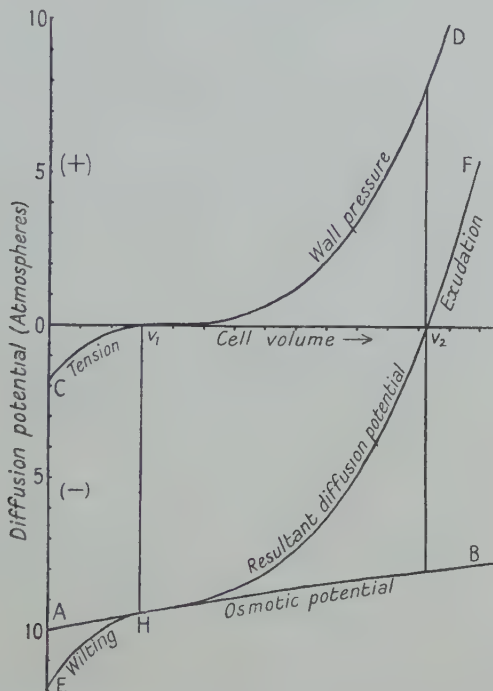


FIG. 2. Diffusion potential diagram of a hypothetical plant cell or tissue.

In Fig. 2 the familiar diagram showing the changes in osmotic pressure, suction pressure, and turgor pressure with increasing cell volume has been modified by using diffusion potential as the ordinate instead of pressure. The abscissa drawn at zero potential represents measurements of cell volume. Above the abscissa the potentials are positive, due to hydrostatic pressures. Below the abscissa the potentials are negative, due either to the presence of solutes or hydrostatic tensions.

The curve *AHB* represents the lowering of the diffusion potential of the cell sap by dissolved solutes (osmotic potential). This curve rises slightly due to the dilution of the solution as the cell volume increases. The curve *Cv₁D* represents the increase or decrease in diffusion potential due to the hydrostatic pressure or tension in the cell caused by the reaction of the cell wall

(wall pressure). This curve has been drawn concave upwards as suggested by Tamiya (1938). *EHF* is the resultant of these two potentials and is the diffusion potential of the water in the cell (suction potential).

At v_1 there is no excess hydrostatic pressure in the cell and the resultant diffusion potential of the cell is due to the dissolved solutes alone, i.e. is equal to the osmotic potential of the sap (incipient plasmolysis). At v_2 the increase of potential due to the hydrostatic pressure in the cell exactly balances the reduction of potential due to dissolved solutes and the resultant diffusion potential is equal to that of pure water—zero. This is the state of full turgidity. If the wall pressure were raised (by some externally impressed force) above its value at full turgidity, the resultant diffusion potential in the cell would become positive as shown to the right of v_2 and water would be exuded from the cell. At points between v_1 and v_2 it is clear that to raise the diffusion potential in the cell to zero (i.e. to cause incipient exudation by external pressure) the wall pressure must be raised by a quantity equal to the resultant diffusion potential, whereas at incipient plasmolysis a pressure equal to the osmotic potential of the cell sap must be applied.

During plasmolysis the excess hydrostatic pressure in the cell is zero, and the resultant diffusion potential equal to the osmotic potential as along *HA*. If the cell loses water by evaporation, on the other hand, the cell wall will tend to be drawn inwards and may even exert a hydrostatic tension in the cell as along v_1C . The resultant diffusion potential in the cell now becomes lower than the osmotic potential as along *HE*.

SUMMARY

An attempt is made to clarify the relationship between turgor pressure and wall pressure by considering the forces acting in a physical model of the plant cell. It is concluded that the turgor pressure and wall pressure are numerically equal in dynamic as well as static conditions.

A diagram showing the water relations of the plant cell in terms of diffusion potential is presented. This has the advantage of making a clear distinction between the separate effects that hydrostatic pressure and dissolved solutes have on the diffusion potential of the cell sap.

I wish to thank Dr. J. H. Davis of the Department of Physics, University of Nottingham, for his critical advice on the preparation of this note.

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The Dynamics of Cell Expansion by Turgor

BY

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ABSTRACT

The writer is now in full agreement with the views of Thoday.

I AM now in full agreement with all the arguments put forward by Thoday (1951), Weatherley (1951), and Spanner (1951) (above) and with all their views and conclusions. Although Spanner has proved my point that the turgor pressure, taken (as heretofore) as the mean hydrostatic pressure, at least theoretically minutely exceeds the wall pressure during cell expansion, the attitude of my note (1951) in which the possibility of a somewhat greater excess was imagined was certainly obfuscated and due to an erroneous visualization of some of the circumstances. I now fully agree with Thoday's view. I would certainly also support Thoday in his suggestion that the term 'turgor pressure' should be retained for the pressure making for turgor, since its importance is a mechanical one. I would, however, define it as the outwardly directed pressure exerted by the contents on the wall, and therefore not make it synonymous with the hydrostatic pressure. In this sense it will be exactly equal to the mean hydrostatic pressure only at equilibrium, but will always be exactly equal and opposite to the inwardly directed pressure due to the tension in the wall. This wall pressure will be the reaction to it and as such will keep exactly in step with it. If I may now answer my own question 'What makes the cell expand?', it is the superior diffusion potential of the water outside. This causes inward movement of water against the viscous resistances to flow. The water which enters increases the hydrostatic pressure and simultaneously the wall pressure, increases in hydrostatic pressure being impossible without exactly corresponding increases in the reacting wall pressure. On this picture there is no question of the viscous resistances to flow opposing adjustment between the contents and the wall; the hydrostatic pressure is entirely offset by the wall pressure and not partially offset by that necessary to move water against the resistances. It is therefore at all times exactly equal to the wall pressure.

I fully agree with Thoday on all scores and am most grateful to all contributors for the very helpful analyses and arguments prompted by the ill-founded attitude of my earlier note.

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An Epiphytic American Species of *Tubicaulis* Cotta¹

BY

SERGIUS H. MAMAY

With Plate IX and three figures in the Text

ABSTRACT

A new species of the coenopterid genus *Tubicaulis* (*T. scandens* Mamay) is described; the description is based upon a single coal-ball specimen from the Upper Pennsylvanian of Illinois. This is the first reported occurrence of *Tubicaulis* in American fossil floras.

The type specimen apparently grew as an epiphyte on a *Psaronius* trunk, with its roots penetrating into the tissues of the support, thereby providing a firm anchorage. It was a long, slender rhizomatous plant, with long internodes and certain dorsiventral anatomical features; in these aspects it presents some interesting points of contrast with previously described species of *Tubicaulis*.

INTRODUCTION

ALTHOUGH more than a century has lapsed since Cotta (1832) proposed the generic epithet *Tubicaulis* to accommodate certain Paleozoic fern-like fossils, comparatively little light has since been shed upon this interesting genus (now usually defined in the restricted sense applied by Stenzel in his classical revision, in 1889). Indeed, it still remains perhaps the least understood of all the Paleozoic genera which Seward (1910) included within the *Ceopteridae*. Despite extensive investigations of English and European Paleozoic floras, only three species of *Tubicaulis* have been reported prior to this communication; the most recent description was that of *T. Berthieri*, by C. E. and P. Bertrand, in 1911.

In 1909 P. Bertrand drew attention to the presence of epiphytic *Tubicaulis* specimens, preserved *in situ* among the roots of silicified *Psaronius* trunks from Chemnitz. Sahni, in 1931, reported epiphytic specimens of *Tubicaulis* as well as other coenopterid genera to be rather frequently encountered on *Psaronii* from both Chemnitz and Bohemia. In neither of these publications were the tubicaulids specifically identified. In a later paper, however (1935), Sahni published a sketch illustrating the epiphytic association; the *Tubicaulis* present in this particular instance was at least tentatively referred to the species *T. Berthieri* C. E. and P. Bertrand.

It thus becomes apparent that *Tubicaulis* embraces two widely divergent forms of plants; large upright sub-arborescent plants with thick armours of

¹ An investigation completed at Cambridge University during the author's period of appointment to a John Simon Guggenheim Fellowship (1950-1).

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persistent leaf bases, as typified by *T. solenites* (Sprengel) Cotta, and slender, creeping epiphytes which very likely found ideal conditions for mechanical support among the tangled masses of adventitious roots surrounding the *Psaronii* upon which they grew. Whether this assemblage actually constitutes a natural genus exhibiting widely diversified ecological adaptations, or whether it represents a group of unrelated species with certain anatomical similarities resultant from homoplastic evolutionary processes, cannot be decided at present, since nothing whatsoever is known of the foliage or fructifications; the vascular organization is of an extremely simple type which might conceivably have been developed independently by two unrelated groups. However, in the complete absence of information concerning those organs of primary phylogenetic importance, i.e. the foliage and fructifications, it seems most feasible to assign to the genus *Tubicaulis* all specimens which share the following combination of anatomical features:

1. An essentially terete, protostelic axial vascular system with exarch structure, and lacking in secondary thickening.
2. Petiole traces which depart from the stele as flat tangential bands and ultimately assume the shape of a C, with the convex surface facing toward the axis.

It should be kept in mind that the above characters present a dependable taxonomic criterion *only when they appear together*. The terete protostele itself is of widespread occurrence among fossil plants, and the inversely oriented C-shaped petiolar trace is known to occur in at least one other genus (*Asterochlaena dubia* Stenzel). In this instance, however, the stellate form of the stele appears to provide sufficient basis for a generic distinction.

The specimen which will be described presently possesses the anatomical characters outlined above and is therefore referred to the genus *Tubicaulis*. It appears to constitute a new species of this genus, and to the best of my knowledge represents the first known occurrence of this genus in American fossil floras. Besides contributing to our knowledge of the geographical and geological distribution of the genus, it provides an additional element of floristic comparison between the American and European Paleozoic. Furthermore, it entails certain anatomical features which indicate a dorsiventral adaptation resulting from the epiphytic habit; this point will be discussed in subsequent pages.

HISTORY OF *TUBICAULIS*

The three previously described species of *Tubicaulis* are as follows:

1. *T. solenites* (Sprengel) Cotta. 1832 (the type species). The single specimen upon the basis of which this species was described, consisted of a silicified trunk from the Lower Permian of Saxony. It was first included in Brongniart's genus *Endogenites* (*E. solenites* Sprengel, 1828), along with a rather heterogeneous group of other unrelated entities. In 1832 Cotta proposed the

generic name *Tubicaulis*, including within the genus as he understood it, several species; of these all but *T. solenites* were removed from *Tubicaulis*, and assigned to other genera by Stenzel in 1889. Stenzel's monograph included the first detailed description of *T. solenites*; his figures are frequently encountered in paleobotanical literature. *T. solenites* was apparently a small erect tree-like form. Its slender stem contained a stele approximately 7 mm. in diameter, and was surrounded by a thick mantle of persistent leaf bases and adventitious roots. The fragment was about 40 cm. high with a basal diameter of 14 cm.; it is not known what proportion of the entire plant this represents.

2. *T. Sutcliffii* Stopes. 1906. This species was based upon a single specimen which occurred in a calcareous roof nodule found in the Bullion Coal Seam in Lancashire, England. Geologically, then, it is the oldest known species. Although the general proportions of this specimen were somewhat smaller than those of the type species, it appears to have been a plant very similar to the latter, in both construction and habit. Small sporangia were found associated with the stem fragment, but there seems to be no valid reason to assume that they were borne upon this plant.

3. *T. Berthieri* C. E. and P. Bertrand, 1911. This species was based upon a single silicification from the Lower Permian of Autun, France. Although far less complete than either of the two previously described specimens, it quite obviously constitutes a distinct species, judging from certain details of the stele and cortex. As previously pointed out, at least one epiphytic specimen was assigned to this species by Sahni in 1935.

At this point it seems appropriate to mention that P. Bertrand, in his classification of the zygopterids (1909), included *Asterochlaena* (*Menopteris*) *dubia* Stenzel within the genus *Tubicaulis* (*T. dubius*). This view has not, however, been supported by subsequent authors (Scott, 1920; Hirmer, 1927).

ORIGIN OF THE AMERICAN SPECIMEN

The specimen was found in a small coal ball collected at the Ralph Brian farm, about two miles north-east of Berryville, Illinois. Although the exposure of this coal-ball deposit is limited to a few yards along the bank of a small stream, material collected here has thus far produced some highly gratifying specimens, among them the remarkably complete specimen recently described as *Botryopteris trisecta* (Mamay and Andrews, 1950). This outcrop lies in the Calhoun horizon of the McLeansboro group (Upper Pennsylvanian). Thus the specimen is somewhat younger, geologically, than *T. Sutcliffii*, and older than both *T. solenites* and *T. Berthieri*.

The uncut coal ball measured approximately 14 cm. in length, 10 cm. in width, and 6 cm. in thickness. Four preliminary saw cuts were made and the orientation of the specimen thus determined. Since the original cuts sectioned the specimen in somewhat oblique planes, two additional cuts were made in order to obtain perfectly transverse surfaces for study. The specimen was investigated by means of the Parlodian peel method. First a few

peels were made from each of the following oblique surfaces exposed by the initial saw cuts; the bottom (*b*) surfaces of sections A, B, C, and D. Then the following series of transverse sections were made: 70 peels from the top surface of section A, involving a total thickness of 24 mm.; 40 peels from surface B-2 (*t*), involving a thickness of 13 mm.; 60 peels from surface C-1 (*b*), involving a thickness of 22 mm.; and 20 peels from surface C-2 (*t*), through a thickness of 10 mm. Two series of longitudinal sections were made: 35 peels from section B-1, and 40 from section E. In order to arrive at an accurate understanding of such details as petiolar departure, root emission, &c., the distances between successive peels in each of the transverse series were made as uniform as possible. The distance involved between peels of series A (*t*), B-2 (*t*), and C-1 (*b*) was about $\frac{1}{3}$ mm.; in series C-2 (*t*) it was 0.5 mm.

The reader is now referred to Text-fig. 1, which represents a profile view of the coal ball (WCB 686); the orientation of the specimen, the planes of sectioning, and the portions of the specimens used in peel series are fully indicated.

TUBICAULIS SCANDENS, sp. nov.

General Description

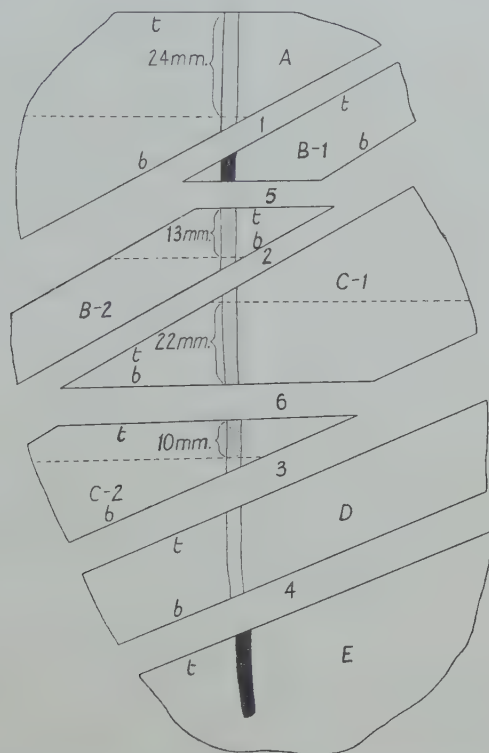
Approximately one half of the bulk of the coal ball was found to consist of a single well-preserved fragment of the inner root zone of a *Psaronius* stem, which extends through the entire length and width of the coal ball. Its rootlets follow a course roughly parallel to the long axis of the coal ball. As a matter of terminological convenience, the psaronean fragment will be referred to as the *support* throughout this paper.

The *Tubicaulis* specimen consists of a slender unbranched stem fragment with a total length of about 13 cm. It follows a nearly straight course through the coal ball, roughly parallel to the long axes of the psaronean rootlets. Lying between the *Tubicaulis* stem and the surface of the support is a dense mat of small adventitious roots. Many of these originate from the *Tubicaulis* specimen; some have penetrated into the tissues of the support for a distance of nearly 2 cm., and must have provided a very firm attachment to the latter when the *Tubicaulis* was alive. No roots are evident on the surface of the *Tubicaulis* which faces *away* from the support. Petiole traces depart from the stele in a rather lax spiral phyllotaxis, with relatively long internodes. The first impression given by this specimen is, then, that of a long, slender, rhizomatous epiphyte; further evidence in support of this assumption will be presented in the detailed descriptions which follow.

The Stem

Cortex. Although portions of the cortex are rather fragmentary and incompletely preserved, enough of it is present to provide several structural details which indicate a fairly complex type of cortical organization with some quite distinctive features.

The cortex consists of two distinct zones. The outer cortex is composed of a compact, homogeneous tissue which is in large part fragmentary and does not completely surround the stem in any single section. This layer of tissue averages about 2 mm. in thickness. Its component cells are nearly isodiametric, with diameters ranging from 50 to 140 μ . They are closely packed and



TEXT-FIG. 1. A profile diagram of coal ball No. WCB 686, illustrating the orientation of the specimen and the planes of sectioning. By oblique cuts 1, 2, 3, and 4 the orientation of the stem was determined; cuts 5 and 6 were made in order to expose transverse surfaces for study. Solid black shading indicates portions used in making longitudinal peel series; stippling indicates portions used for transverse peel series; millimetric measurements indicate the lengths involved in transverse series; broken lines indicate the level of the last peel of each series. Unshaded portions of the specimen are preserved in the Washington University paleobotanical collection.

have relatively thick walls. The largest cells are comparatively few and are irregularly interspersed among the smaller ones. No evidence of an epidermis or any sort of indument has been seen. The outer cortex is shown in Pl. IX, Fig. 1.

Because of its fragmentary nature and the fact that the outer cortex is in large part flattened in a plane parallel to the surface of the support, it is difficult to determine the total diameter of the stem with any degree of

certainty. Judging from the dimensions of the outer cortical fragments, however, it seems reasonable to assume that the gross diameter of the stem did not exceed 2 cm.

The inner cortex contains three distinctive features.

The most conspicuous of these consists of large, irregularly shaped but discrete patches of dark-brown cells with dense contents, which are randomly distributed throughout the length of the specimen. These nests are elongate in the direction parallel to the axis of the stem, and when seen in transverse section (Pl. IX, Figs. 6 and 10) they vary from nearly circular to very irregular shapes, and reach a millimetre or so in transverse dimensions. From 5 to 8 such nests may be seen in almost any section of the stem. Since the component cells are usually well preserved and sharply defined, while the tissue immediately surrounding them may be completely lacking in preservation, it is not likely that these nests were formed as the result of decomposition processes. They more probably represent a system of specialized cortical cells which were present in the living plant. The cells are closely packed, somewhat angular in cross-section, and reach transverse dimensions of approximately 90μ . Their lengths range to 200μ . They are uniformly filled with a dark-brown substance which serves to readily distinguish them from the surrounding cortical matrix. Their walls are slightly thicker than those of the surrounding cortical parenchyma.

The true nature and function of these nests can only be conjectured; they may represent sclerotic nests which were preserved prior to full lignification of the cell walls. On the other hand, they may represent patches of cells which were either of a secretory nature or specialized for storage of food reserves. Somewhat similar structures were found in the cortex of *T. Berthieri*; to these was attributed a 'glandular' nature.

The second distinctive feature of the inner cortex lies in a system of well-defined nests of large, thin-walled parenchymatous cells which are devoid of contents (see Pl. IX, Fig. 8). The individual cells are consistently larger than those of the dark-brown nests described in the preceding paragraph, reaching diameters of 200μ and lengths of 350μ . As shown in Pl. IX, Fig. 15, these patches of cells may be quite elongate parallel to the stem axis. Here again, any attempt to explain the function of these apparently specialized cortical cells would be purely conjectural.

The tissue which composes the remainder of the inner cortex is extremely delicate and hence its preservation is sporadic. It consists of a system of large, thin-walled cells with diameters up to 170μ , interspersed among smaller ones with diameters usually less than half as great. This tissue is usually best preserved in the areas immediately surrounding the vascular system (see Pl. IX, Figs. 6 and 11), and presents a rather 'spongy' appearance. The organic continuity between this ground tissue and the compact outer cortical layer has been noted in some portions of the specimen.

Vascular organization. The xylem is a simple solid mass of primary wood, appearing roughly elliptical in cross-section throughout the greatest portion

of the specimen. Only for a short distance in the A (*t*) peel series does it display the nearly circular aspect which is typically brought to mind in connexion with *Tubicaulis*. In this particular portion its diameter is about 2.8 mm. Throughout the remainder of the specimen, however, the xylem maintains a more or less elliptical outline which is distorted here and there by protuberances produced by the departing petiole traces and roots, and by flattened facets which form above the nodes as a result of abstriction of the petiolar xylem mass (see Pl. IX, Figs. 1, 6, 12, and 14; also Text-fig. 2). The dimensions vary from 2.5 to 3.5 mm. in width, and from 2 to 2.3 mm. in thickness. In any particular section the xylem is roughly 50 per cent. greater in width than in thickness (with the exception of the short distance mentioned above). It is important to note that the stele is consistently oriented so that its greatest transverse dimension (i.e. the *long* axis of the ellipse) lies essentially *parallel* to the surface of the support. As an added measure of convenience in terminology, the surface of the stele *facing* the support will be referred to as the *ventral* surface, that facing *away* from the support will be termed the *dorsal* surface, and the opposite surfaces which are separated by the greatest distance will be termed the *lateral* surfaces. While the primary purpose of this terminology is one of convenience, there are certain aspects of the specimen which appear to justify it from the morphological point of view. Particular attention will be paid to these points as they arise.

Without first making a thorough examination of the stele, one might logically reason that its asymmetrical form was caused by crushing, with the surface of the support representing the bedding plane. Ordinarily this might account for the consistency with which the dorsal and ventral surfaces of the stele parallel the surface of the support. But it would be mechanically impossible for a terete strand to be crushed into the asymmetrical form displayed through the greater portion of this specimen, without causing a collapse of the tracheidal walls, or at least a distortion of the shapes of a noticeable proportion of the tracheids. While such distortion of tracheids is visible in a number of the sections, it is not present in sufficient amount to fully explain the asymmetry of the stele. In the greater proportion of the sections there is *no evidence* of crushing of the tracheids. The sections shown in Pl. IX, Figs. 1 and 14, convincingly illustrate the tracheids in their fully distended natural shapes, throughout the stele. This, along with additional evidence to follow, precludes the conclusion that the elliptical shape of the stele is a function of mechanical distortion. Furthermore, it lends support to the interpretation of this plant's symmetry as dorsiventral rather than radial.

Although stelar measurements vary somewhat at different levels in the specimen, there is no distinct tapering from one end to the other. The dimensions of the most apical portion are slightly less than those at the most basal transverse sections, but these variations in size are no greater than those which occur in positions intermediate between the two extremities.

The xylem is exarch. When seen in transverse section (Pl. IX; Fig. 14), the tracheids show sharply angular, polyhedral outlines. The largest elements

are about 280μ in diameter, as compared with diameters of 15 or 20μ in the smallest. The largest tracheids lie towards the centre of the xylem mass, and the smallest ones at the periphery, with a gradual gradation between the two extremes. The smallest tracheids are almost entirely confined to small concentrations which lie below the points of departure of petioles and roots. These elements depart with the outgoing appendages. As shown in Pl. IX, Fig. 14, the greater portion of the stelar surface is apparently devoid of elements which can be definitely identified as protoxylem.

The tracheidal walls are very thin; the thickness of the combined walls of two adjacent tracheids rarely exceeds 20μ . In the most terminal portion of the specimen (A-t series) the walls show a delicately sinuous outline. This does not, however, distort the natural shape of the cells, nor cause the facets to lose their identities. It occurs equally in all directions, and in combination with the extreme thinness of the tracheidal walls, seems to suggest shrinkage of but scarcely lignified tracheids, rather than crushing.

The phloem is poorly preserved. It appears as a conspicuous dark band of badly disorganized tissue surrounding the xylem, reaching a thickness of 250μ in some sections. Structural details are almost entirely lacking. Here and there, however, there are suggestions of phloem cells in radial alinement.

An endodermis is not distinguishable.

Details of tracheidal pitting were investigated in two complete series of longitudinal sections taken at intervals of about 50μ . The tracheids vary in length, those with the greatest diameters reaching 7 mm. The ends taper gently and uniformly, terminating in acute points. Pitting ranges from scalariform to reticulate. Although particular attention was paid to the pitting of the narrowest peripheral tracheids, none were found with spiral or annular thickenings. Secondary thickening in the walls of the narrowest elements is of a simple scalariform type (Pl. IX, Fig. 3), with the closely set (about 3μ apart) transverse bars about 3μ wide. Connexions between the bars are very delicate and sparse. There is no evidence of the protoxylary stretching which occurs in these elements in plants with reasonably rapid rates of growth. Stopes (1906) noted a similar condition in the xylem of *T. Sutcliffii*; this may well be interpreted as evidence that both *T. scandens* and *T. Sutcliffii* were plants with extremely slow growth.

The largest tracheids have multiseriate-reticulate wall sculpturing, with four to six longitudinal rows of simple pits on each facet. The pits, which are transversely oriented, range from 5 to 8μ in length, from 2 to 4μ in width, and are separated by bars of thickening about 3μ in width (Pl. IX, Fig. 2).

Transverse septation of the tracheids was observed in one instance, occurring immediately below the point of departure of a root from the stele. As seen in Pl. IX, Fig. 5, a few of the peripheral elements are divided into short segments by essentially transverse septa. The tracheidal walls, as well as the septa, display the characteristically reticulate sculpturing, although in these elements it is not so pronounced as that of the adjacent unsegmented

elements. This peculiar feature was also reported to occur in the xylem of *T. Berthieri*.

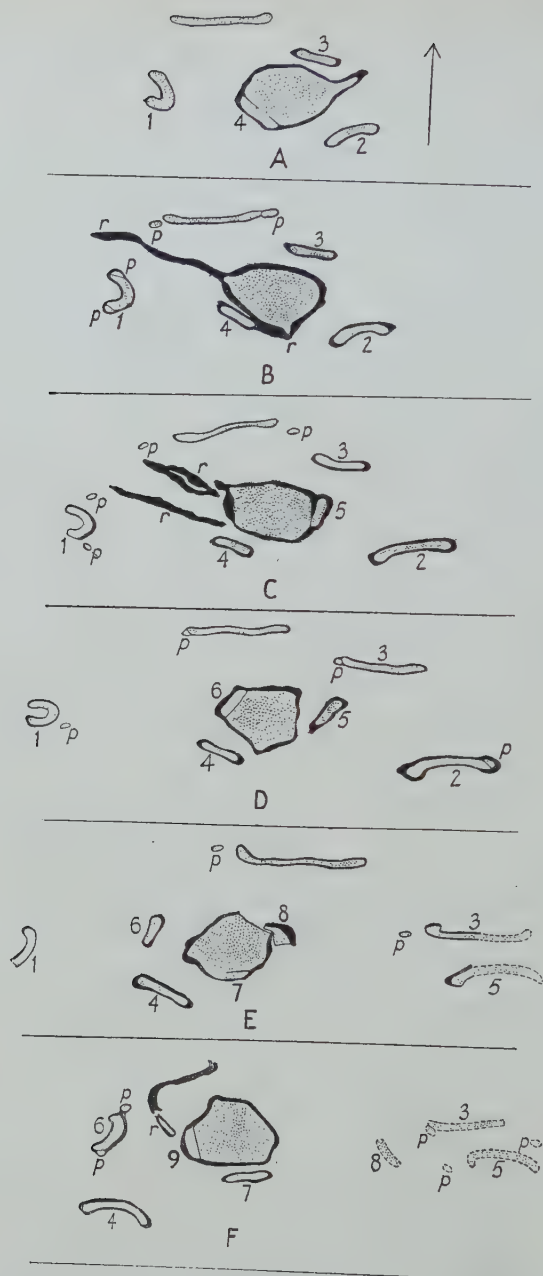
The Petioles

The emission of a petiole trace from the stele is first indicated by a small concentration of narrow tracheids which extends tangentially across a portion of the stele at a depth of 0.4 mm. or less (Pl. IX, Fig. 6). These small elements and the larger ones external to them are destined to constitute the xylem of the foliar trace. Within a millimetre or so above this stage, this segment of the xylem is separated from the stele, accompanied by a gradual encroachment of the dense black tissue (presumably the phloem) which closely invests the stele. When first separated, the foliar trace, as seen in transverse section, has the form of a straight, narrow band, ranging from 1 to 1.2 mm. in length and from 0.25 to 0.3 mm. in width (Pl. IX, Fig. 14). The entire adaxial portion may leave the stele simultaneously or one side may slightly precede the other; there is no evident regularity in this feature. The separated trace is completely surrounded by a thin sheath of phloem remains.

Angles of departure vary somewhat, depending upon the portions of the stele from which the petioles depart. Those originating from the lateral surfaces usually show a greater angle of departure than those departing from the dorsal or ventral surfaces. In the case of a 'lateral' petiole observed in the longitudinal series E, the trace was approximately 2.5 mm. distant from the stele at a point 14 mm. above its node of origin; the angle involved is roughly 15 degrees.

The petioles which depart from lateral position show a marked tendency to twist slightly as they travel outward, eventually lying with their adaxial surfaces facing a plane extending through the lateral surfaces of the stele (see Text-fig. 2). This occurs while the petiolar traces are still within the cortex of the stem. It is difficult to state whether the variability in angles of departure and the petiolar torsion are wholly ascribable to mechanical pressure, or whether they represent the manifestation of dorsiventral tendencies of this plant. It seems most reasonable to attribute them to a combination of both factors.

Following their departure from the stele, the petiolar traces undergo a gradual increase in width and thickness. Simultaneously the sides gradually bend towards each other and away from the stele. This results in the characteristic form of the *Tubicaulis* foliar strand: a shallow gutter-like or C-shaped band of xylem, with the *convex* surface facing *towards* the axis. The increase in size during this differentiation of the petiole trace may be as much as four times the size of the strand as it first departs from the stele; widths of nearly 4 mm. and thicknesses of nearly 0.5 mm. are exhibited by some of the traces. This differentiation is usually quite marked within a distance of 2 cm. above the point of departure from the stele. With one exception, all the petiole traces observed seem to lie well within the cortex of the stem; hence the curvature of these traces is not very pronounced. It is, however, sufficiently



TEXT-FIG. 2. An ascending series of camera lucida outline drawings illustrating various stages of departure of nine successive petiole traces. Diagram A is taken from surface C-2 (*t*), and the remainder from the C-1 (*b*) peel series, with the exception of the portions indicated by broken lines, which were present in surface B-2 (*b*). The vertical distances between successive stages are approximately as follows: A-B, 2 mm.; B-C, 4 mm.; C-D, 3 mm.; D-E, 6 mm.; E-F, 6 mm. Traces are numbered according to their positions within the spiral, No. 1 being the lowest, and No. 9 the highest. The arrow in diagram A points in the direction of the *Psaronius* fragment.

Xylem is stippled; solid black shading indicates phloem remains. *p* = pinna trace; *r* = root.

marked in every instance that it may be ascertained without question that the curvature develops with the *convex* side *towards* the axis.

As the petiolar xylem becomes detached from the stele, the protoxylem elements decrease abruptly in number, and at the point where the separation first becomes complete, the only evidence of protoxylem is shown by a group of two or three small elements at each end of the band, slightly towards the adaxial surface. As the petiole trace then proceeds outwards (Pl. IX, Fig. 11) small tracheids are seen to be sparsely scattered over its entire adaxial surface. The remainder of the trace is composed of two or three ranks of large elements which are roughly oblong in cross-section, with their greatest transverse dimensions lying perpendicularly to the ab- and adaxial surfaces of the trace.

Only one completely isolated petiole was observed. Its trace forms a nearly semicircular arc with a linear distance of 2.5 mm. between the ends. This petiole is surrounded by rather distorted remains of a dense outer cortex; within this the xylem is invested by the peculiar 'spongy' parenchyma described in the stem cortex. This petiole is roughly 6 mm. in diameter, taking into account the distortion of the outer cortex.

Since it was not possible to follow the course of any single petiolar trace for a distance of more than 5 cm., the ultimate curvature of the trace is not known.

The internodes are long in proportion to the stelar dimensions. Throughout the peel series they range from 4 to 6 mm. in length, with two exceptions. In these instances (Text-fig. 2, E; Pl. IX, Fig. 1) two consecutive traces are shown departing almost simultaneously. Although the section shown in Pl. IX, Fig. 1, was prepared from one of the original oblique surfaces (section B (b) 8), the angle of obliqueness was not sufficiently great that the plane of the section would intercept two distant nodes.

The petioles depart in a clockwise spiral which entails, on the average, a vertical distance of about 1 cm. for the completion of one revolution. The angles of divergence, however, cannot be measured accurately because of the asymmetrical form of the stele. They appear to lie somewhere between the extremes of 120 and 180 degrees. Consequently the phyllotaxy cannot be accurately established. Although it seems to closely approximate a 2 : 5 ratio, the fact that the petiole traces show a marked tendency to originate from lateral positions on the stele suggests the probability that the ratio involved is actually more complex. Of seventeen petiole traces observed departing from the stele, only four were initiated from positions well within the dorsal or ventral surfaces, the remainder originating from lateral positions.

In Text-fig. 2 is presented an ascending series of outline drawings of sections taken from 2 to 6 mm. apart. This illustrates the departure of nine successive petiole traces and the changes undergone during their course through the cortex of the stem.

Pinna Traces

The first pinna traces are usually initiated within a centimetre of the point of separation of the petiole trace, and consequently while the latter is still

within the stem cortex. They are formed in essentially opposite pairs, first appearing as small bulges at the edges of the *adaxial* surface of the petiole trace. The angles and direction of departure appear to be quite variable, although they usually proceed in directly opposed courses away from the sides of the parent strand. Although this point is not entirely clear, the pinna traces seem to be of *monarch* structure. When first separated they are small oval strands measuring about $150 \times 250 \mu$ in transverse dimensions, with a concentration of very small tracheids on the side facing the stem stele (Pl. IX, Figs. 7, 11). The largest tracheids do not exceed 50μ in diameter. As the pinna traces proceed outward, the distinction in size of the tracheids gradually becomes less sharp, until they are uniformly tiny in diameter. Meanwhile the trace gradually assumes the form of a straight narrow band; this may be a stage preparatory to the division of the strand, as it is known to occur in *T. solenites*, or it may represent a duplication in miniature of the form of the parent petiolar strand. This point remains unsolved, as the traces ultimately disappear entirely, beyond this stage. Their extrastelar organization is not known.

In one instance, three successive pairs of pinna traces departed within a few millimetres of each other; two consecutive pairs were found departing from each of several other petiole traces.

It is difficult to arrive at a satisfactory interpretation of these traces. Their origin from the petiolar strand while the latter is still within the cortex of the stem, and the gradual decrease in diameter of their tracheids cast doubt upon the possibility of their having functioned as the vascular supplies of free lateral appendages of the petiole. However, the alternative of interpreting them as roots is precluded by the following points of contrast:

1. Whereas the roots contain large metaxylem elements (to 150μ in diameter) from the very outset, the largest elements of the pinna traces are extremely small at their points of origins and gradually decrease in size, suggesting increasing proximity to the growing-point.
2. Whereas the pinna traces originate with *monarch* structure, the clarity of which is soon lost, the roots exhibit *diarch* structure, which is clearly seen in even the smallest root steles.

Roots

The roots provide a considerable aid in the understanding of the ecological conditions under which the specimen grew, and in interpreting its morphological adaptations.

Perhaps the most significance lies in the manner in which the roots depart from the stele. (No roots are known to originate from the petiole traces.) Particularly close attention was paid to this feature through the entire series of transverse sections. In the 7 cm. of length thus studied, 30 roots depart from the stele. Although there is no apparent regularity of root departure with reference to the nodes, it is significant to note that of the 30 roots

observed, only *one* departs from the dorsal surface of the stele; the remainder originate from the ventral and lateral surfaces, and *all* follow courses directed *towards* the support. This condition is by no means suggestive of a plant with radial symmetry; it seems to most convincingly support a dorsiventral interpretation of the specimen.

In transverse section the roots first appear as small protuberances on the surface of the stele, measuring approximately 0.5 mm. across their bases. They follow a somewhat basally directed path at first, then they proceed laterally or directly towards the support and soon become hopelessly entangled among the numerous roots which cover the surface of the latter (Pl. IX, Fig. 4). This mass of roots reaches a thickness of a centimetre at some points.

The roots vary from 1 to 2 mm. in diameter. The cortex is composed of closely packed, thick-walled cells. There is a gradual decrease in size of cortical cells, the smallest ones lying in the innermost layer (Pl. IX, Fig. 13).

The endodermis is sometimes seen as a dark line surrounding the stele; cellular details are lacking, however. In the better-preserved roots very delicate phloem cells, which do not exceed 15μ in diameter, may be seen surrounding the xylem.

The xylem is diarch. In the largest roots the xylem strands are nearly terete, reaching 0.5 mm. in diameter, and containing as many as 20 tracheids from 15 to 150μ in diameter. The protoxylems are most conspicuous in the smaller roots, in which the xylem is typically more elliptical in transverse section (Pl. IX, Fig. 13).

Dichotomous branching of the roots has been observed.

The psaronean root fragment, or support, is excellently preserved. There is scarcely any sign of decay in the filamentous tissue surrounding these roots. The extrastelar tissues lying within the sclerotic sheaths which delimit the individual psaronean rootlets are, however, partially disorganized or nearly absent; prior to fossilization this must have resulted in a system of essentially hollow tubes, occupied by the *Psaronius* root steles.

Some of the *Tubicaulis* roots have penetrated into the tissues of the support, in some instances to a depth of 2 cm. or more. Of these, many lie within the psaronean root cavities (the hollow tubes mentioned in the preceding paragraph), as many as three or four occupying the same one. In one instance a *Tubicaulis* root was followed for a distance of more than 2 cm., throughout which distance it was intimately appressed to the stele of a psaronean root. Several tiny *Tubicaulis* rootlets were observed within the psaronean root cavities, with root-hairs intact and in their undistorted natural positions (Pl. IX, Fig. 9).

It is difficult to say whether the *Psaronius* roots as a whole were functional immediately before being subjected to conditions of fossilization. In some the xylem appears intact, while in others it is in various stages of disorganization. However, it seems highly probable that the *Psaronius* was alive at the time of penetration of the *Tubicaulis* roots, for two chief reasons. Firstly, there is little evidence of pre-fossilization decay in the delicate filamentous

matrix which comprises the bulk of the inner root zone. Secondly, the fact that the great bulk of the *Tubicaulis* roots are on the *surface* of the support would seem to imply that the latter was actually a firm, living tissue at the time of intrusion, resisting penetration to such an extent that comparatively few *Tubicaulis* roots succeeded in burrowing very far into the support. This evidence suggests a true epiphytic association. Furthermore, it seems likely that more than one, and possibly several, epiphytes grew on this single *Psaronius* trunk, as the mass of epiphytic roots is far out of proportion to that which one might expect to be produced by a single plant as small as the specimen under discussion. The great abundance of *Psaronii* in the Berryville flora has been previously pointed out (Mamay and Andrews, 1950), and it is hoped that future collections of this excellent material will reveal additional information of the kind presented above, and provide a more complete insight into the ecological conditions which prevailed when this interesting flora flourished.

COMPARISON WITH PREVIOUSLY DESCRIBED SPECIES

Notwithstanding the limitations of comparison imposed by the scantiness of the material which has provided the basis for previously described species, the American specimen appears to represent a distinct new species of *Tubicaulis*, for reasons which will be presented shortly. However, before discussing its points of contrast with other species, it seems fitting that we summarily review the conclusions of primary importance which may be drawn concerning *Tubicaulis scandens*, along with the supporting evidence.

1. It appears to have been a long, slender, rhizomatous plant. The slenderness of the stele, with its relatively long internodes, suggests that we are dealing with a representative portion of a possibly much longer plant which was mechanically incapable of supporting itself in an erect position.
2. In all likelihood it represents an epiphytic species. Sahni's observations of epiphytic tubicaulids have already been pointed out. Although details concerning the degree of intimacy of these associations are not known, the roots of the American specimen burrowed into the apparently living tissues of the psaronean support, thereby providing a firm anchorage for its slender rhizome.
3. It exhibits certain dorsiventral features. The elliptical outline of the stele cannot be explained as a function of crushing (compare Pl. IX, Fig. 6, which shows some evidence of crushed tracheids, with Pl. IX, Fig. 14, in which no sign of mechanical distortion of the tracheids is apparent). This, along with the tendency for lateral initiation of foliar traces, indicates a morphological dorsiventrality. Furthermore, the almost complete absence of root initiation on the *dorsal* surface of the stele may be interpreted as a physiological dorsiventrality and a further point in support of such a morphological interpretation.

A comparison of *T. scandens* with *T. solenites* illustrates the most conspicuous difference between the two species to lie in the comparative sizes of their steles and petiole traces. In both species the petiole traces, measured at the points of departure from the stele, are roughly 1 mm. wide and 0.25 mm. thick. The diameter of the stele of *T. solenites*, however, reaches 7 mm. or about twice the greatest diameter of that of *T. scandens*. Thus the proportional bulk of xylem involved in the formation of petiole traces is much greater with reference to the stele in *T. scandens* than in *T. solenites*.

The pinna traces present a second difference between these two species. In *T. scandens* they depart from the petiole traces very close above the nodes, while the petiole traces are still within the cortex of the stem; in *T. solenites*, however, there are none given off until the petiole is entirely separate from the stem. In final contrast, *T. solenites* shows none of the rhizomatous, dorsiventral aspects of *T. scandens*; it was a radially constructed, upright form.

The English species *T. Sutcliffii* differs from *T. scandens* in several features. Its stele (about 2 mm. in diameter) and component tracheids (to 150μ in diameter) are somewhat smaller than those of *T. scandens* (stele attaining dimensions of 2.3×3.5 mm.; tracheids to 280μ in diameter). Furthermore, while Stopes reported adventitious roots originating from foliar traces as well as the stele in *T. Sutcliffii*, root initiation was confined to the stele in *T. scandens*. Finally, the absence of dorsiventral anatomical features in *T. Sutcliffii* provides in itself a sound basis for separating these two species.

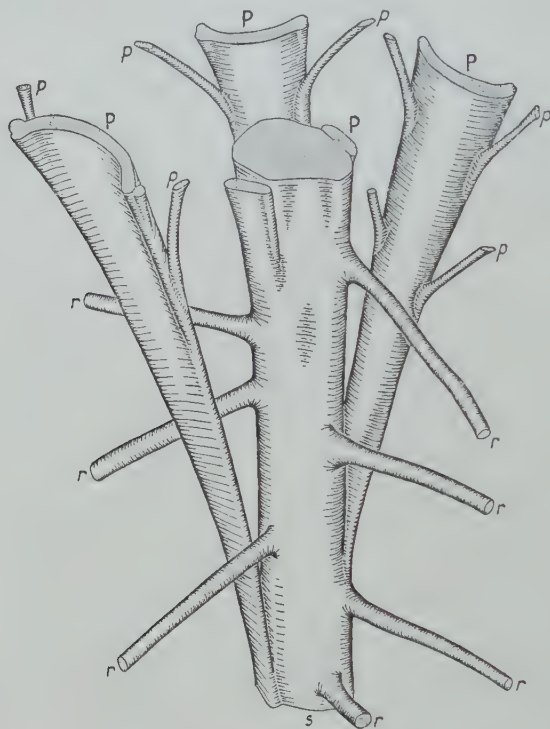
It is unfortunate that more is not known concerning the French species, *T. Berthieri*. While the type material was apparently exquisitely preserved, it was limited to six thin sections, and consequently some of the more important details of its structure are not known. In a few general aspects, such as size of the stele and petiole traces, and the asymmetrical form of the stele, it bears a close resemblance to *T. scandens*, a likeness to which additional weight is provided by a consideration of Sahni's previously mentioned identification of an epiphytic specimen as *T. Berthieri*. However, a comparison of the cortical tissues provides reason to consider *T. scandens* to be specifically distinct from, although probably closely related to, *T. Berthieri*. The peculiar 'spongy' inner cortical organization of *T. scandens* is absent in *T. Berthieri*; and although the cortex of the latter species contains scattered groups of specialized (glandular?) cells, they are not consolidated into large conspicuous nests such as are present in *T. scandens*.

The distinctive features of *T. scandens*, which is so named in reference to its habit, are summarized in the diagnosis which follows. A reconstruction of the vascular system is presented in Text-fig. 3.

SPECIFIC DIAGNOSIS: *TUBICAULIS SCANDENS*, sp. nov.

Plant with a long, slender, rhizomatous stem, apparently unbranched. Stele varying in outline from nearly circular to irregularly elliptical, with transverse dimensions ranging from 2.0×2.5 to 2.3×3.5 mm., the greater

dimension consistently parallel to the surface of the substrate (in this case a psaronian fragment). Tracheids from 15 to 280μ in diameter, the smallest at the periphery. Narrowest tracheids scalariform; widest tracheids multi-seriate-reticulate. Protoxylems sparse and irregularly distributed, most abundant at points of root and petiolar departure.



TEXT-FIG. 3. Reconstruction of the vascular system, viewed from the *ventral* surface of the stele. Length of the internodes slightly reduced in order to conserve space. *r* = root; *p* = pinna trace; *s* = stele; *P* = petiole trace.

Outer cortical tissue composed of compactly crowded, thick-walled cells. Inner cortex containing large, conspicuous nests, some composed almost entirely of large (to $200 \times 350\mu$) thin-walled, empty cells, others with smaller (to $90 \times 200\mu$) cells filled with a dense brown substance. Inner cortical ground tissue delicate, 'spongy', composed of large (to 170μ diameter) thin-walled cells interspersed among much smaller ones.

Internodes of varying length, usually 4 to 6 mm. long. Petiole traces departing in a clockwise spiral, with varying angles of divergence and a tendency to originate from lateral positions. Petiole traces with basal dimensions of $1-1.2 \times 0.25-0.30$ mm., gradually increasing to widths of 4 mm. within 5 cm. of the nodes. Greatest known curvature of petiolar traces approximately semicircular. Protoxylems inconspicuous, restricted to two

small lateral groups at base of trace, but sparsely distributed over adaxial surface higher up.

Pinna traces departing in essentially opposite pairs from sides of adaxial surface of petiole traces, the lowest pair initiated within a centimetre of the node. Pinna traces small (to $150 \times 250 \mu$ in transverse section), oval, with monarch xylem; division unknown.

Roots initiated from stele only, and predominantly from ventral surface; roots to 2 mm. in diameter, with diarch steles to 0.5 mm. in diameter.

AGE: McLeansboro group of the Upper Pennsylvanian.

LOCALITY: Berryville, Illinois.

HOLOTYPE: A single specimen, preserved in the Washington University paleobotanical collection as five fragments of coal ball No. W.C.B. 686 (preserved portions indicated in Text-fig. 1), ten series of peels, and slides No. 1870–1905, inclusive.

DISCUSSION

There is little that can be said concerning the affinities of *Tubicaulis*. In its simple anatomical structure it contains certain points of similarity to the genus *Grammatopteris* Renault, which Sahni (1932) postulated as a link between the Osmundaceae and Zygopterideae. Hirmer (1927), however, allied the latter genus with *Botryopteris* Renault, placing *Tubicaulis* in a separate group (Tubicaulidaceae) of the Botryopteroidae. Although Sahni disagreed with the implied relationship between *Botryopteris* and *Grammatopteris*, he supported the segregation of *Tubicaulis* as an isolated entity. Since the grounds for classification of *Tubicaulis* are presently limited to a few fundamental anatomical features, it is apparent that any attempt to evaluate its systematic position in relation to other coenopterid genera must be considered speculative and depend upon discoveries of the foliar and reproductive organs for further corroboration or refutation. Unfortunately *Tubicaulis scandens* provides no information whatsoever concerning these important criteria. Its primary interest lies rather in the information it provides concerning the morphological plasticity within this group of species which, judging from our limited knowledge of them, appear to be closely related, and in the ecological data provided by the specimen.

SUMMARY

1. The fossil plant described in this paper belongs to the genus *Tubicaulis* Cotta. It was found in a coal ball from Berryville, Illinois, and represents the first known occurrence of this genus in American fossil floras.

2. The specimen consists of part of a long, slender, apparently unbranched rhizome with dorsiventral anatomical features. Petiole traces depart in a lax spiral, and predominantly from lateral positions on the stele. The petiole traces give off small pinna traces in essentially opposite pairs. Root initiation

is almost entirely restricted to the ventral surface of the stele. Foliage and fructifications are unknown.

3. The specimen apparently grew epiphytically on a *Psaronius* plant. Some of its roots form part of a dense mass of roots on the surface of the psaronean fragment, while others burrowed into the tissues of the *Psaronius* to depths of 2 cm. or so.

4. Comparison with previously described species of *Tubicaulis* indicates the American specimen to represent a new species. The specific name *Tubicaulis scandens* is proposed.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to Dr. H. Hamshaw Thomas, F.R.S., for many kind and helpful criticisms offered during the preparation of this paper, and to Dr. H. N. Andrews, Jr., for the loan of the specimen from the Washington University palaeobotanical collections. Thanks are also due to Mr. F. T. N. Elborn of the Botany School, Cambridge University, for the preparation of the photographic illustrations.

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EXPLANATION OF PLATE IX

FIG. 1. Slightly oblique transverse section of the stele, showing two petiole traces departing nearly simultaneously. A root initial is shown at the right; a fragment of the outer cortex appears at the upper part of the photograph. Slide no. 1876. $\times 6.5$.

FIG. 2. Longitudinal section of a metaxylem tracheid, showing the reticulate thickening of the wall. Slide no. 1877. $\times 360$.

FIG. 3. Longitudinal section of a protoxylem tracheid, with scalariform thickening. Slide no. 1877. $\times 580$.

FIG. 4. Transverse section showing the epiphyte and support. *Tubicaulis* roots are seen in varying depths of penetration into the *Psaronius* fragment. Note the orientation of the dorsal-ventral stele with respect to the support. Slide no. 1870. $\times 3.5$.

FIG. 5. Longitudinal section of the xylem below a root initial showing transverse septation of tracheids. Slide no. 1879. $\times 120$.

FIG. 6. Enlargement of the section shown in Fig. 4. The stele is surrounded by five petiole traces; a sixth is about to depart from the ventral (upper right) surface, as indicated by the concentration of small tracheids extending tangentially across the stele. A pinna trace is departing from the left-hand margin of the petiole trace at the top of the photograph. Dark patches surrounding the stele are nests of specialized cortical cells with dense contents. Note the thin layer of phloem surrounding the xylem, and the 'spongy' appearance of the cortex. Slide no. 1870. $\times 11$.

FIG. 7. Transverse section of a petiole trace, from which a pair of pinna traces is departing. Curvature of the petiole trace has apparently been accentuated by mechanical distortion, as evidenced by the crushed tracheids at the centre of the arc. Monarch structure is clearly shown in the pinna trace at the upper right. Slide no. 1899. $\times 27$.

FIG. 8. A nest of large, thin-walled, empty cells found in the inner cortex. Compare with the nest shown in Fig. 10. Slide no. 1872. $\times 25$.

FIG. 9. Transverse section showing a small *Tubicaulis* root, sectioned through its root-hair zone, and lying within the cavity formed by disorganization of the extra-stelar tissues of one of the roots of the psaronean fragment. Note the root-hairs. To the left of the epiphytic root may be seen the remains of the psaronean root stele. Slide no. 1971. $\times 16$.

FIG. 10. A nest of inner cortical cells with dense contents. Several of these may be seen in Figs. 4 and 6. Slide no. 1872. $\times 34$.

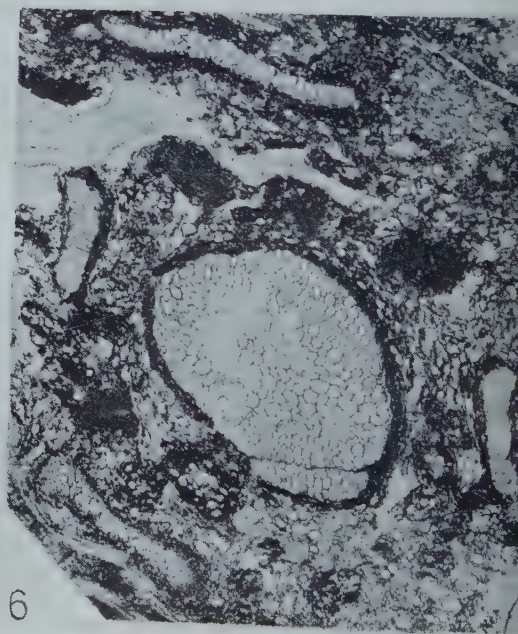
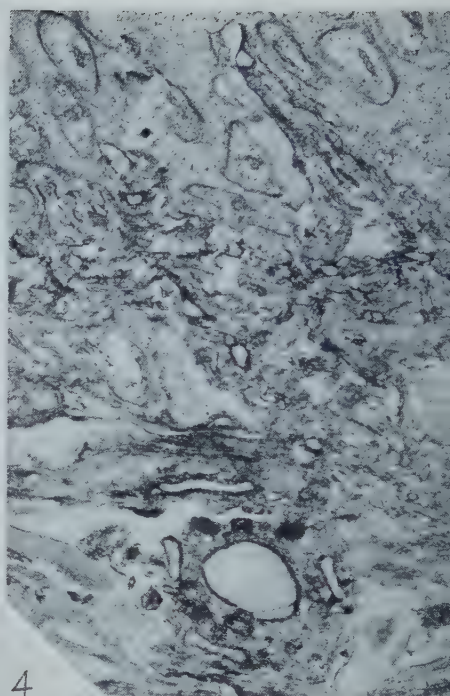
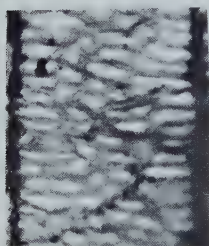
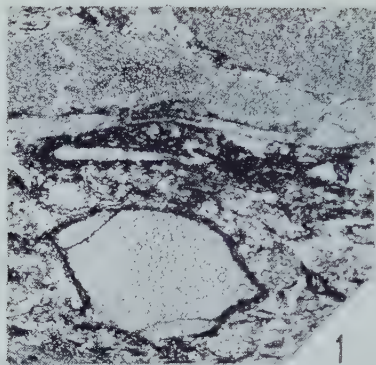
FIG. 11. Transverse section of a petiole trace, with a pinna trace departing from the lower end. Note the 'spongy' cortical parenchyma surrounding the trace, and the monarch structure of the pinna trace. Slide no. 1873. $\times 18$.

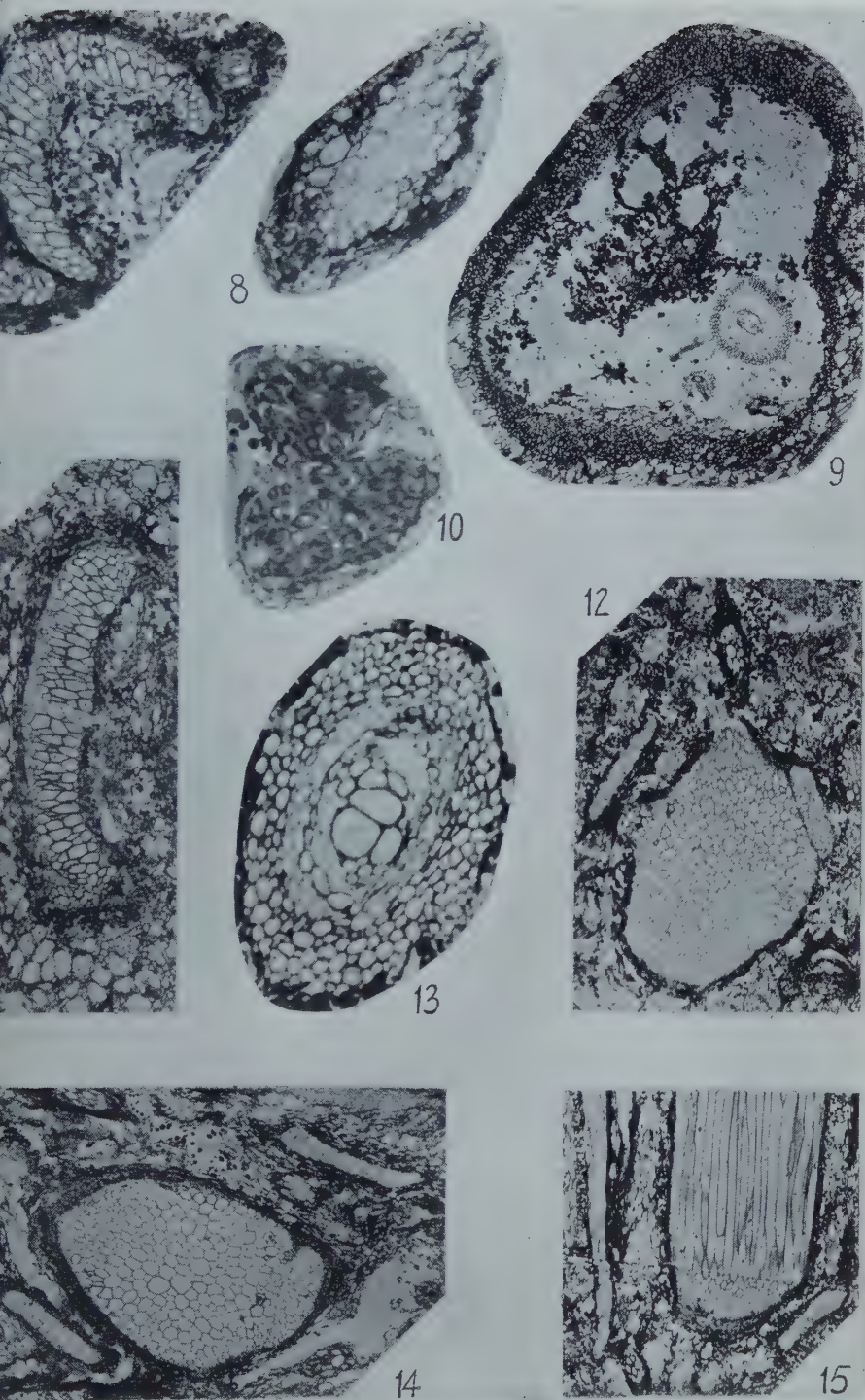
FIG. 12. Transverse section of the stele, showing departure of two roots, one towards the top of the photograph, and one from the left-hand (ventral) surface. A petiole trace is about to depart toward the right. Slide no. 1875. $\times 11$.

FIG. 13. Transverse section of a root, showing the diarch stele and compact cortical tissue. Slide no. 1870. $\times 54$.

FIG. 14. Transverse section of the stele, with a petiole trace about to depart toward the right. Note the asymmetrical outline of the stele, and the fully distended, uncrushed tracheids. Slide no. 1899. $\times 11$.

FIG. 15. Three-dimensional (transverse-tangential) section of the stele, with two closely associated petiole traces. An elongate nest of thin-walled, empty cortical cells is shown between the stele and the petiole trace at the left. Slide no. 1880. $\times 11$.





Experimental and Analytical Studies of Pteridophytes

XVII. The Effect of Various Physiologically Active Substances on the Development of *Marsilea* in Sterile Culture

BY

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With Plates X and XI and six Figures in the Text

ABSTRACT

A technique for growing *Marsilea* aseptically is described. The effects of varying concentrations of indoleacetic acid, α -naphthaleneacetic acid, and 2,4-dichlorophenoxyacetic acid have been studied—they resemble in general those reported for other plants. Additions of adenine, guanine, or uracil have no observed effects.

The effects of departures from complete nutrition and of additions of growth-promoting substances are described and discussed in relation to observed changes in leaf morphology.

INTRODUCTION

IN contrast to the impressive record of observed fact relating to the normal development of organs and tissues in all the principal groups of vascular plants, there is as yet but little knowledge of the morphogenetic factors involved. During recent years, however, two valuable approaches have been opened up by the discovery of plant growth-regulating substances and the development of methods of sterile culture of plant organs and tissues. Both of these aspects of plant growth have been discussed in several recent reviews, e.g. Audus (1949), Gautheret (1942, 1945), Thimann (1948), White (1943, 1946, 1949).

The responses of the tissues of intact plants to the action of growth substances have been the subject of considerable study (Thomson, 1945), but comparatively little work has been done on the effect of physiological concentrations of these substances on organs or tissues growing in sterile culture. The methods of sterile culture also provide an opportunity for a more direct study of the effects of nutrition on general morphology and tissue differentiation than was formerly possible.

In the present and subsequent papers the methods outlined above will be applied to certain representatives of the Pteridophyta, a group which has received little attention from workers in the fields of plant hormones and tissue culture. The present contribution deals with the growth of *Marsilea* in

sterile culture, and with the effects of certain physiologically active substances on the growth and early development.

MATERIALS AND METHODS

Most of the experimental work was carried out on *Marsilea Drummondii* A.Br., an Australian species. In some experiments *M. vestita* Hook. and Grev. was also used.

Preparation of sterile cultures. The sporocarps of species of *Marsilea* are hard and resistant. There is consequently little difficulty in sterilizing the surface of the sporocarps. This was usually effected by immersing the sporocarp in absolute alcohol for half an hour, followed by a 5-minute treatment in saturated bromine water. The sporocarp was then washed once in sterile water and transferred to a Petri dish also containing sterile water. Natural opening of the sporocarp is very erratic and may take a long time. To ensure immediate opening it is necessary to nick the wall with a sharp scalpel. The subsequent liberation of the spores and development of the gametophytes has been described in detail in various standard works, e.g. Campbell (1918), Bower (1926), Eames (1936), Smith (1938).

Development of the spores is very rapid. When the opening sporocarps are placed in an incubator at 24° C., fertilization occurs overnight, and by 18 hours from the initial splitting of the sporocarp development is usually so far advanced that the small embryo can be clearly seen through the transparent tissue of the female prothallus. At this stage in development the 'spores', each with its attached embryo, were transferred to tubes or Petri dishes containing the culture medium.

Culture media. The basic medium was similar to that recommended by Gautheret (1942) and unless otherwise stated had the following composition:

Knop's solution (full strength)	.	.	.	.	.	.	.	500 ml.
Glass distilled water	.	.	.	.	.	.	.	500 ml.
Berthelot's solution (modified)	.	.	.	.	.	.	.	10 drops
Glucose (A.R.)	.	.	.	.	.	.	.	30 g.
Thiamin hydrochloride	.	.	.	.	.	.	.	0.001 g.

The thiamin was omitted in certain cultures without any obvious effect on the amount of growth, but since it had been used in the initial experiments it was retained throughout the present work. Other substances were added according to the requirements of the experiment. Media prepared in this way have usually a pH of approximately 6.0. Otherwise they are adjusted to this value by addition of a few drops of dilute sulphuric acid or potassium hydroxide solution. The media were sterilized by autoclaving at 110° C. for 20 minutes.

Since the present investigation dealt principally with the early stages of development, most of the cultures were grown in glass tubes of 15 × 1.8 cm. containing 10 ml. or 15 × 2.5 cm. with 20 ml. of medium. The tubes were capped with cellophane with the object of preventing excessive evaporation while permitting some gaseous interchange.

Conditions of growth. The cultures were maintained in a culture room with the temperature regulated at $21^{\circ} \pm 1^{\circ}$ C. and with a single 'daylight' fluorescent lamp (5 ft. 80 W.) suspended above the cultures as the sole source of light. As described later, this light was sufficient to avoid any appearance of etiolation in the sporelings, but was inadequate for growth unless an organic carbon source was provided in the medium. Measurements indicated a light intensity of 100 f.c.s. at the level of the cultures, but the sporelings in the tubes would receive only a fraction of that value.

Experimental observations were based on a study of either six or twelve cultures per treatment. The effects of different concentrations of a substance were investigated using sporelings produced by the same sporocarp, and one batch from each sporocarp was always grown on the normal glucose medium as a control. Development in each batch was very uniform and only occasional aberrant sporelings were obtained.

EXPERIMENTAL RESULTS

Development of sporelings on glucose media

In the first attempt at growing *Marsilea* in sterile culture the spores after fertilization were transplanted to the normal medium stiffened to a gel with 1 per cent. agar. It is well known that many plants grow well under such conditions, but the *Marsilea* sporelings grew very slowly when cultured in this way. Fairly well developed agar cultures of the two species are shown in Pl. X, Figs. 1 and 2. Parallel cultures in liquid media showed much greater growth as clearly illustrated by Pl. X, Figs. 3 and 4.

The better growth of *Marsilea* in liquid media is in contrast with that reported for many other plants and may be related to its semi-aquatic natural habitat. But this aspect was not studied in detail, since liquid cultures were more suitable for the work in hand.

Liquid cultures. The development of the sporeling in the basic liquid medium containing 3 per cent. glucose is quite comparable with that which has been described repeatedly for natural conditions. Several species were studied in detail by Braun as early as 1870. A striking feature is the increase in complexity of the successive leaves. This is illustrated for *M. Drummondii* in Text-fig. 1.

The gradual elaboration of form in the juvenile leaves of many pteridophytes has been cited as an instance of recapitulation (Bower, 1917). Goebel (1900) held the opposing view that the simpler juvenile leaves are arrested formations resulting from inadequate nutritive supply. This belief has received support in the present work from cultures of *Marsilea* in which no organic source of carbon was supplied. In such cultures the limited food reserves of the spore were soon exhausted. Thus, spores which had been fertilized and left in darkness on tap-water developed a first leaf of normal size, but only a very small second leaf and two or three roots. There was no further growth. Cultures grown in liquid media of normal composition but with the omission

of glucose showed rather greater development but produced only simple leaves, and eventually died off. One of these plants is illustrated in Text-fig. 2, the limited root development being particularly noticeable. The light in-



TEXT-FIG. 1. *Marsilea Drummondii*. After 6 weeks in medium containing 3 per cent. glucose and 10^{-8} IAA. Successive leaves are numbered. ($\times 6$.)

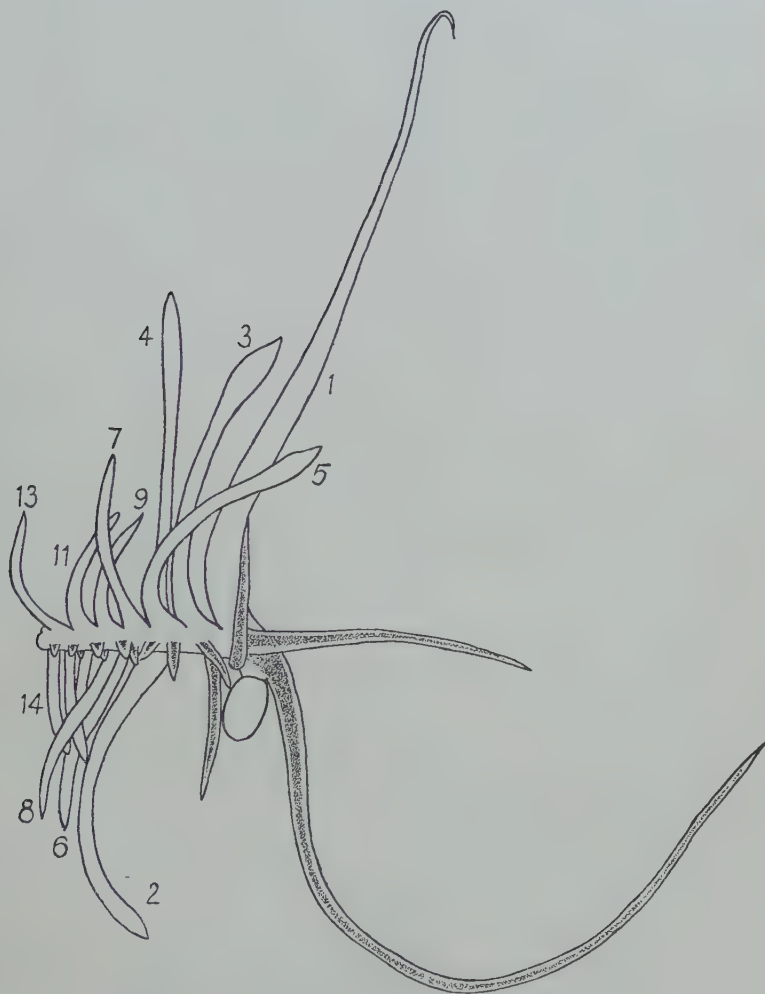
tensity used was evidently insufficient to permit the growth of *Marsilea* in the absence of organic nutrients.

Growth in darkness. Vigorous and apparently unlimited growth occurred in complete darkness in liquid media containing glucose as the sole organic constituent. The sporelings showed the usual symptoms of etiolation (Pl. X, Fig. 5) and were almost colourless with only traces of chlorophyll in the

reduced laminae of the attenuated leaves. The internodes of the rhizome were greatly elongated and root development was relatively feeble.

The effect of indoleacetic acid

Observations on the effect of adding indole-3-acetic acid (IAA) to the



TEXT-FIG. 2. *M. Drummondii*. After 7 months on basic medium with omission of glucose. Successive leaves are numbered. ($\times 10$.)

medium were based on three series of culture from separate sporocarps. In each series cultures were grown in the dark as well as in light.

In series 30/1 and 16/2, the following concentrations as g./l. of IAA

were made up in basal medium: 10^{-8} , 10^{-7} , 10^{-6} , and 10^{-5} . In series 23/3, concentrations 10^{-7} and 5×10^{-6} g./l. were used.

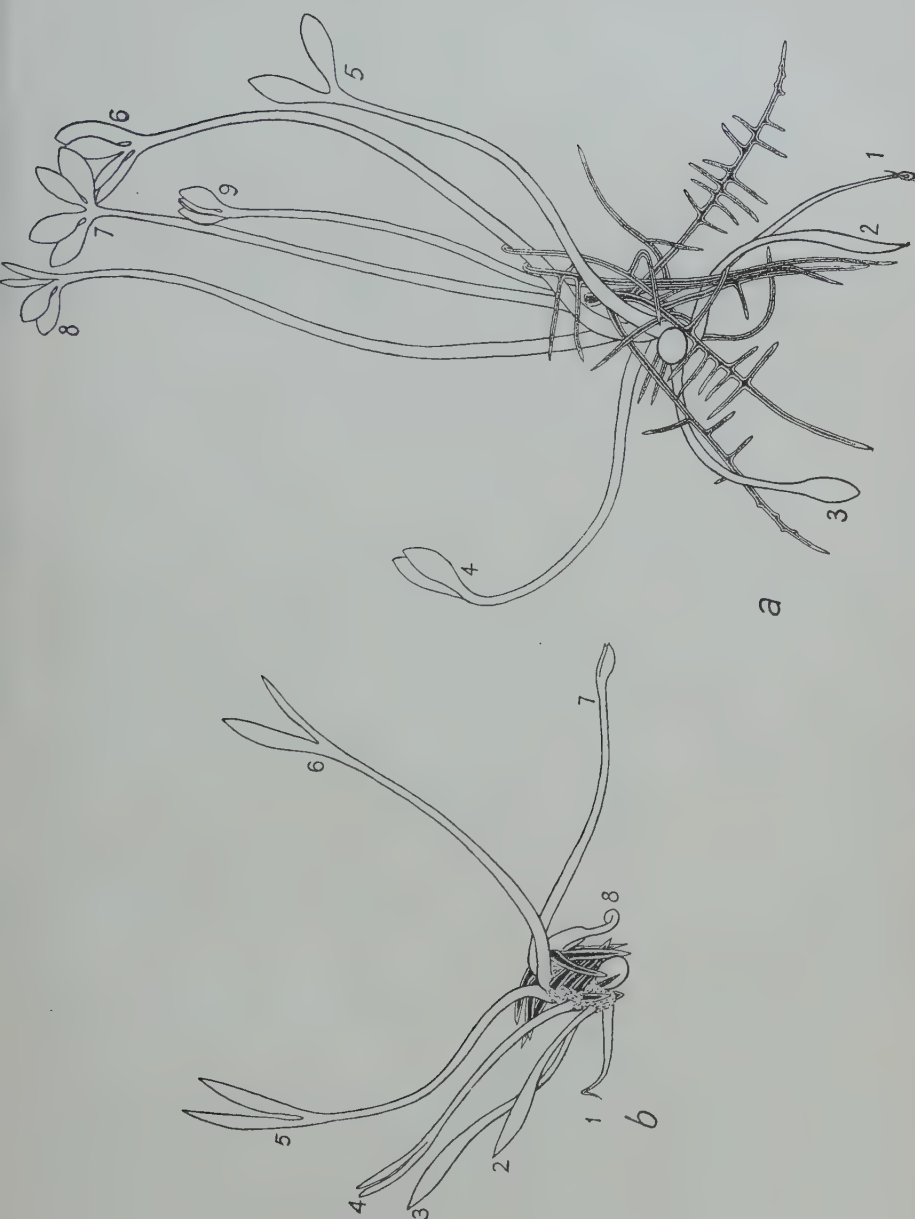
In the three series the results were closely comparable. The lowest concentration, 10^{-8} , appeared to have no effect on the magnitude or nature of the growth. A typical plant from this batch after 6 weeks of growth is illustrated in Text-fig. 1.

In 10^{-7} IAA, root growth was inhibited slightly during the first week but this effect soon disappeared and older plants showed no evident differences from those developing without IAA. Sporelings grown in 10^{-6} IAA, however, showed striking differences from normal. During the first 3 weeks growth in length of the roots was markedly inhibited. After 5 weeks the roots were still shorter than normal but some had developed numerous laterals which as a result of their straightness and close two-ranked arrangement gave a very distinctive appearance to the roots. One of these plants after 6 weeks of growth is shown in Text-fig. 3*a*. A similar root is illustrated in Pl. XI, Fig. 13.

The effect of 10^{-5} IAA was very pronounced. During the first few weeks of growth roots could not be detected by external observation, but examination after 3 weeks revealed a number of very short stout roots. After 6 weeks fewer leaves had been produced than in the three lower concentrations. The leaves were still bifid and with longer narrower lobes than in the normal plants. A typical plant of this series is illustrated in Text-fig. 3*b*. As indicated in the figure, the rhizome was distorted by irregular masses of tissue surrounding the bases of leaves and roots. Further growth was accompanied by a gradual return to a more normal habit. Longer roots developed which at first showed the presence of the numerous laterals characteristic of cultures in 10^{-6} IAA. Finally, as in 10^{-6} IAA there was a complete recovery to apparently normal plants. Sporelings grown in 5×10^{-6} IAA at first resembled those in 10^{-5} but showed a more rapid recovery.

In cultures maintained in the dark similar effects were observed. In 10^{-5} IAA the elongation of the rhizome was at first suppressed, but as in the plants grown in the light there was a gradual recovery to the normal condition, in this case of greatly elongated internodes.

Anatomical effects. A complete anatomical examination was made of a sporeling after 3 months of growth in 10^{-6} IAA. Except in the roots no distinct departure from normal could be detected. But examination of a plant which had been grown for 14 weeks in a solution originally containing 10^{-5} IAA showed a pronounced modification of the structure. Sections through the part of the rhizome developed during the first month or so of growth, as in Text-fig. 3*b*, showed a cellular proliferation of the outer cortical tissues and almost complete suppression of the large air-chambers which run longitudinally in the normal rhizome. A section of this kind is figured in Pl. XI, Fig. 12, while Pl. XI, Fig. 11, illustrates a normal section for comparison. In certain regions cells had expanded to produce the relatively 'giant cells' so frequently described for tissue cultures treated with various growth substances, e.g. Gautheret (1945), Morel (1948). A striking feature was the return



TEXT-FIG. 3. *M. Drummondii*. After 6 weeks in 3 per cent. glucose media containing (a) 10^{-6} and (b) 10^{-5} IAA respectively. Successive leaves are numbered. ($\times 6$.)

to normal in later formed parts of the rhizome, which showed the typical structure with air-chambers and characteristic lateral meristems.

Another effect of IAA after prolonged exposure is revealed by microscopical examination of the roots. Dark swellings on the roots are seen to result from considerable and irregular enlargement of the cortical cells leading to their loosening and separation. This effect is particularly prominent around the base of the laterals, as in Pl. XI, Fig. 13. It has been observed for concentrations of IAA of 10^{-6} , 5×10^{-6} , and 10^{-5} in both light- and dark-grown plants.

The effect of naphthaleneacetic acid

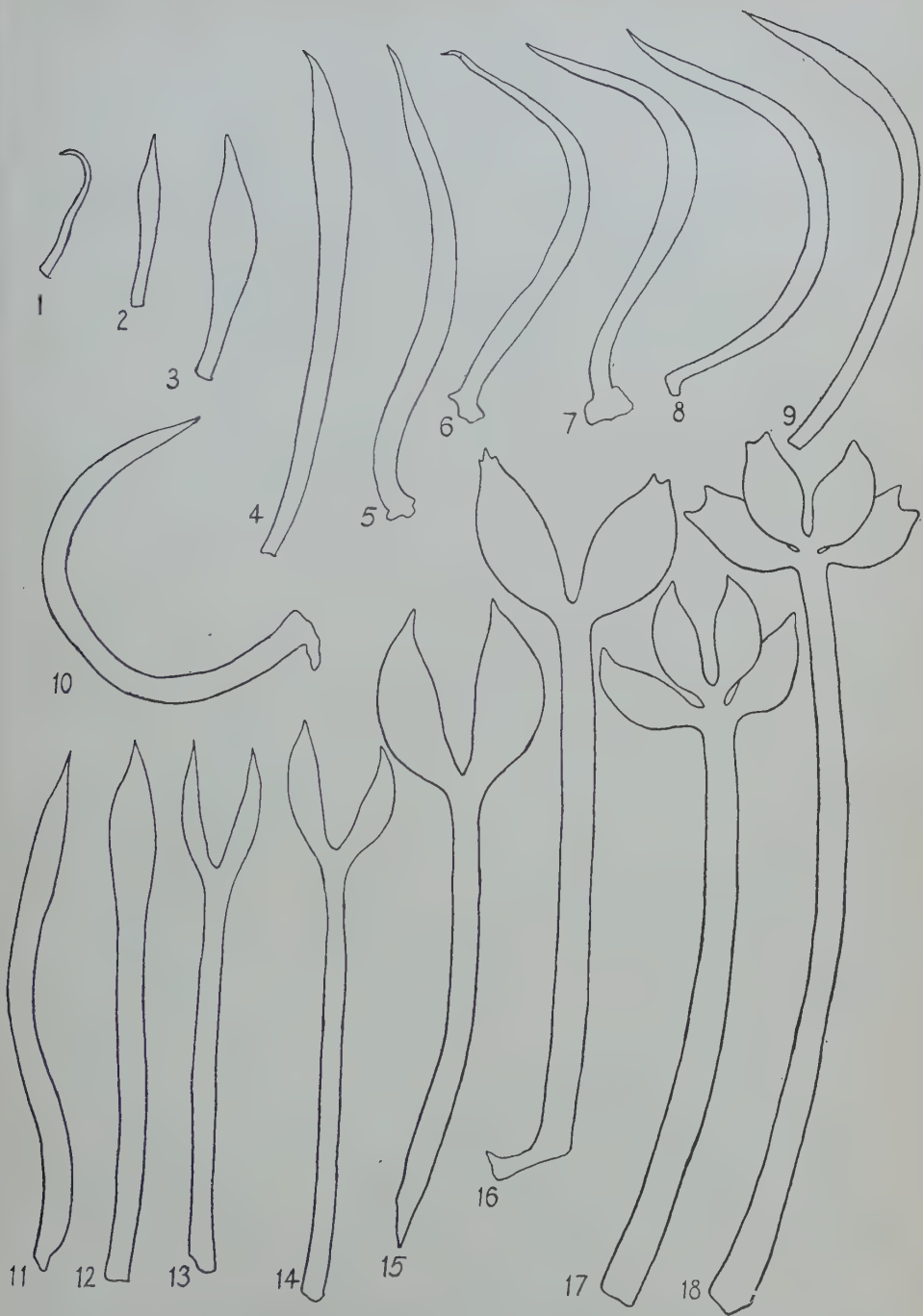
The effects of α -naphthaleneacetic acid (NAA) were studied in three series of cultures. In the first two, 30/1 and 16/2, concentrations of 10^{-8} , 10^{-7} , 10^{-6} , and 10^{-5} g./l. were used. In series 23/3 the concentrations were 10^{-7} and 5×10^{-7} g./l.

There were distinct differences from the effects of IAA. At 10^{-8} development was quite like that of the control on glucose only. In 10^{-7} NAA root development was at first slightly inhibited but the later total growth of roots was greater than in 10^{-8} NAA or in the control and the plants as a whole were rather more vigorous, although these purely quantitative aspects were not studied in detail in the present work. In concentrations of 10^{-6} considerable morphological changes were produced. After a fortnight of growth the short rhizome was strikingly abnormal and had expanded into a tumour-like mass of irregular tissue which appeared bright and shining as a result of the reflection of light. The plants developed slowly and for a period of 2 months, in contrast to those on lower concentrations of NAA, produced only simple leaves which showed a characteristic inward curvature in the direction of the apex. After 12 weeks in the 30/1 series, and 10 weeks in the 16/2 series, the plants had recovered and were producing normal leaves and roots. In 10^{-5} NAA, only a minute conical embryo with no external sign of differentiation developed, although squashing revealed 6–7 minute roots each with its vascular strand. In darkness the effects were similar, and in 10^{-6} NAA the rhizome was at first short and tumorous but eventually recovered to the usual etiolated condition.

In the 23/3 series, normal plants again resulted in 10^{-7} NAA, but sporelings grown in 5×10^{-7} NAA showed the peculiar development of the rhizome already reported for concentrations of 10^{-6} .

A sporeling of this series which had been grown in 5×10^{-7} NAA for a period of approximately 6 weeks is illustrated in Pl. X, Fig. 6. This figure shows four abnormal leaves, short dark inhibited roots, and the peculiar tumorous development reported above. Pl. X, Fig. 7, is an enlarged view of the same sporeling and gives a clearer picture of the hypertrophied rhizome.

Text-fig. 4 records the outlines of successive leaves removed from a plant (series 30/1) which had been cultured for 13 weeks on 10^{-6} NAA. A comparison with any normal plant, e.g. Text-fig. 1, reveals the profound modification in leaf form. The early leaves 1–3 are clearly smaller than usual. Leaves



TEXT-FIG. 4. *M. Drummondii*. Successive leaves from a plant grown for 13 weeks in a medium containing 3 per cent. glucose and 10^{-6} NAA. ($\times 8$.)

1-12 retain a simple outline and are borne on that region of the rhizome characterized by irregular development and by greatly inhibited roots. In leaves 12-17 there is a gradual transition to the normal morphology. In this transitional region the tumorous character of the rhizome is lost and normal roots appear.

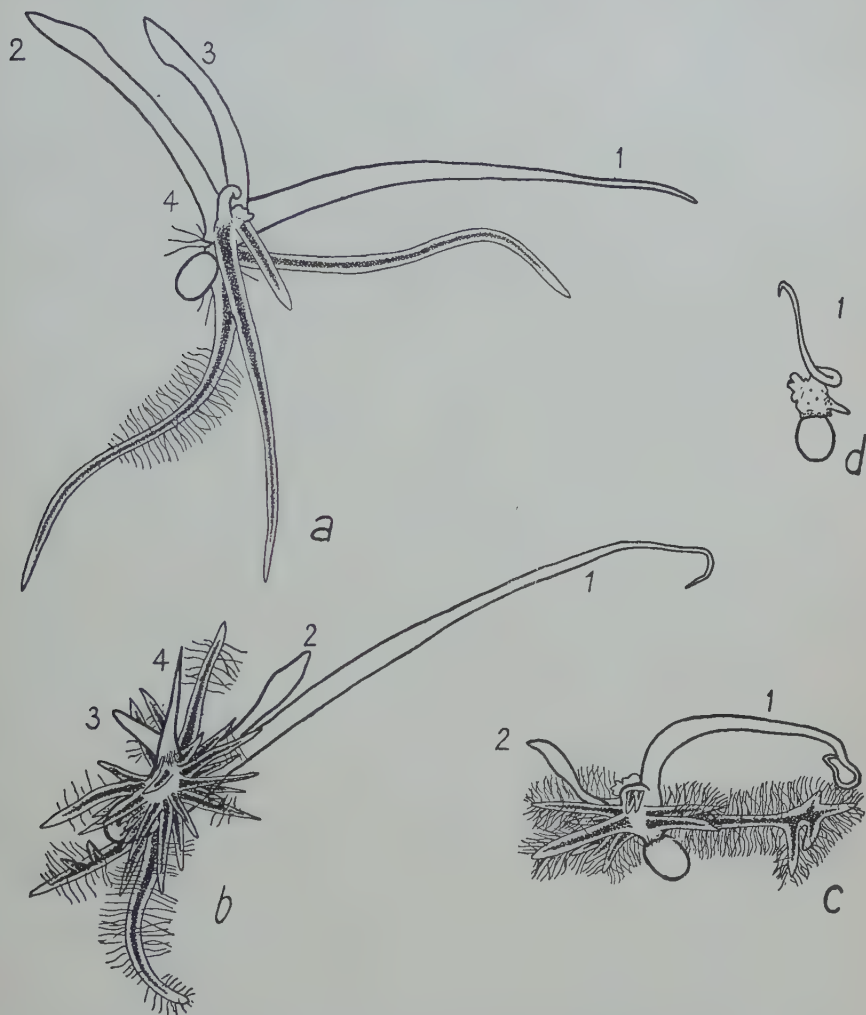
Anatomical effects. Among the effects of NAA the most striking is the hypertrophy of the rhizome to produce a shining irregular tissue when cultured in concentrations of 10^{-6} and 5×10^{-7} . One of a series of section through the rhizome of the sporeling shown in Pl. X, Figs. 6 and 7, is illustrated in Pl. XI, Fig. 14. The serial sections revealed quite clearly that the shining translucent tissue is not a solid callus but is formed by a greatly hypertrophied epidermis which is thrown into prominent bulges enclosing large air-spaces and thus accounting for the bright appearance of the tissue. It is also evident that there is a considerable proliferation of the cortex, the characteristic air-chambers being entirely obliterated. In this abnormal region, inhibited root primordia are present, and one is seen to the lower right of the figured section. The recovery of such plants to a normal external morphology has been described above, and as shown in Pl. XI, Fig. 11, the internal structure of these recovered plants is also completely normal.

The effect of 2,4-dichlorophenoxyacetic acid

Observations on the effect of 2,4-dichlorophenoxyacetic acid (2,4-D) were based on two series of cultures, 15/2 and 23/3. In the first series of cultures concentrations of 10^{-5} , 10^{-6} , 10^{-7} , and 10^{-8} were used. In the second series cultures were supplied with 5×10^{-7} and 5×10^{-8} , 2,4-D.

In the 15/2 series differences were strongly marked after a fortnight of growth and typical plants at this stage are shown in Text-fig. 5. In the 10^{-8} 2,4-D development is comparable with that of the controls. A pronounced effect is seen at 10^{-7} where an abnormal number of roots has arisen, although individual roots are shorter than in the normal plants. There are more root-hairs than usual and one short root shows formation of laterals which are not normally found at this stage. Only the first leaf is of normal dimensions, the second leaf being smaller than in the controls, while leaves 3 and 4 are greatly reduced. At 10^{-6} inhibition of the leaves is more marked and even the first leaf is quite small. Here again there is an abnormally large number of roots, which are all densely covered with long root-hairs. Another abnormal feature found in all cultures in this concentration of 2,4-D is the production of short swollen laterals near the apex of the primary root. A photograph of the original plant of Text-fig. 5 (c) is illustrated in Pl. X, Fig. 8. As shown in Text-fig. 5 (d) only slight growth has resulted in 10^{-5} 2,4-D, and no further development was obtained. Development was also by now at a standstill in 10^{-6} 2,4-D, but in 10^{-7} growth continued for a time. There was little further formation of leaves, and plants consisting almost entirely of roots were obtained. The roots remained unbranched but were covered with long root-hairs.

In 10^{-8} 2,4-D most cultures developed normally but occasional sporelings produced supernumerary roots, which in contrast to those developed at higher concentrations of 2,4-D retained a normal appearance, without excessive



TEXT-FIG. 5. *M. Drummondii*. After 2 weeks on 3 per cent. glucose media containing (a) 10^{-8} , (b) 10^{-7} , (c) 10^{-6} , and (d) 10^{-5} 2,4-D respectively. Successive leaves are numbered. ($\times 6$.)

formation of root-hairs. Following a period of abnormal development such plants reverted to the normal condition after about 2 months of growth. Pl. X, Fig. 10, shows a sporeling of this type after a total growth period of 10 weeks.

In the 23/3 series, cultures of *M. Drummondii* in 5×10^{-7} 2,4-D were very similar in appearance to those already described for 10^{-7} 2,4-D, but differed

in the swelling of some of the root-tips to form relatively large and dark tumorous masses (Pl. X, Fig. 9). In 5×10^{-8} 2,4-D some cultures were indistinguishable from the controls but in other cases there was excessive root development. In 10^{-7} 2,4-D, however, every culture showed a strong rooting response.

In the sporelings which reacted to 10^{-8} and 5×10^{-8} 2,4-D, there was an interesting correlation between excessive root formation and the occurrence of abnormal leaves. This is clearly shown in Text-fig. 6, which represents successive leaves from the plant illustrated in Pl. X, Fig. 10. The first three leaves are typical, while leaves 4–6 although small still retain the usual shape. Leaves 7–10 are distinctly abnormal with certain leaf lobes, represented merely by minute spikes. Leaf 9 is an extreme case in which two small projections are the sole residue of the lamina. Leaf 11 is normal except in size and represents a transition to the normal condition found from leaf 12 onwards.

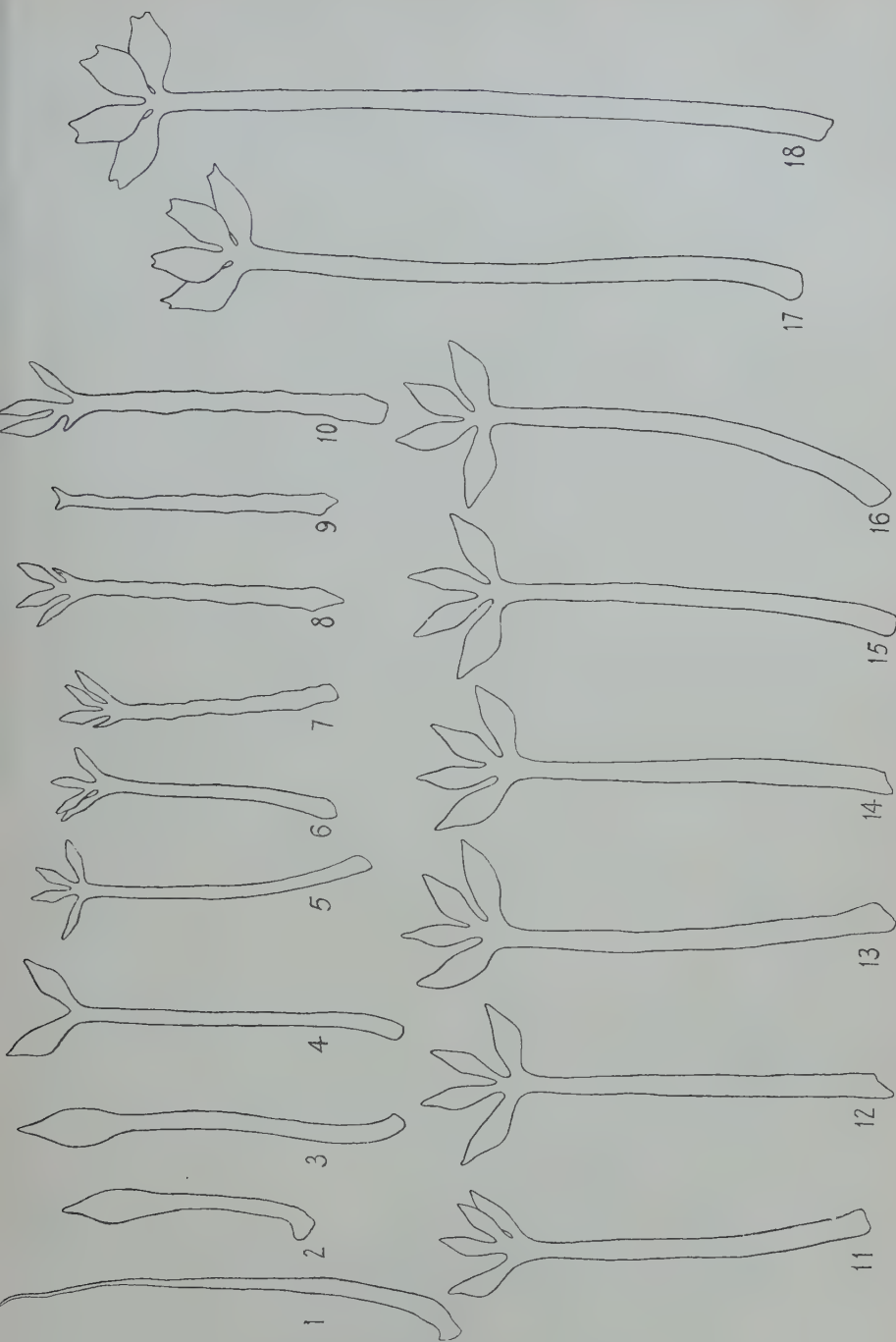
Anatomical effects. Serial sections through the plant illustrated by Pl. X, Fig. 10 demonstrate that, starting from the spore, the structure is at first quite normal, but in the region where extra roots are developed the characteristic air-chambers of the rhizome become occluded. A transverse section in this region (Pl. XI, Fig. 16) shows the origin of roots from all sides of the rhizome, in contrast to the usual arrangement in which at any node the roots arise below a leaf on one side of the rhizome only. Later sections reveal a transition to the typical structure of a normal plant. In the abnormal region fasciated roots are common and this feature is seen on the right of the figured section.

Sections through plants grown in 10^{-7} 2,4-D show pronounced deviation from the normal structure, which is completely distorted by the exuberant root formation. In addition to the great number of roots externally visible, the cortical tissues are riddled with undeveloped root primordia. Pl. XI, Fig. 15, illustrates a longitudinal section of the plant figured in Text-fig. 5 (b). In this case numerous root primordia have been differentiated even in the parenchymatous tissue immediately behind the apex, and other sections show some roots arising from the tissue in the base of the petioles.

In cultures maintained on 10^{-7} 2,4-D for a period of 11 weeks certain roots showed a characteristic dark thickening. A photomicrograph of such a root is shown in Pl. XI, Fig. 17, and demonstrates that the thickening is a result of expansion and loosening of the cortical cells similar to that already reported for higher concentrations of IAA. The figure also illustrates the abnormal production of root-hairs, which extend even to the apex of the root. Roots from cultures in 5×10^{-7} 2,4-D also show expansion and loosening of cortical cells, but the more definite tumours are a result of distinct proliferation of the apical meristem.

The effect of purine and pyrimidine bases

In view of the importance recently attached to purine compounds as factors in leaf growth and in the differentiation of tissue-cultures (see Discussion), it seemed of interest to study their effect on the development of *Marsilea*.



TEXT-FIG. 6. *M. Drummondii*. Successive leaves from a plant grown for 10 weeks in a medium containing 3 per cent. glucose and 10^{-8} 2,4-D. ($\times 6$.)

In one series of cultures, 16/2, parallel experiments were conducted using media containing adenine, guanine, and uracil at concentrations of 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} g./l. In the cultures containing guanine, development was uniform at every concentration. With uracil only the highest concentration, 10^{-4} , produced any effect and this was restricted to moderate inhibition of growth in length of the roots. The inhibition gradually disappeared and after several months all the cultures were alike. No differences were obtained in the cultures containing adenine.

In view of this result the adenine experiment was repeated. Cultures 7/3 were supplied with adenine at concentrations of 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} g./l., while cultures 23/3 received 2×10^{-4} , 10^{-4} , and 10^{-6} g./l. In a few cases the early leaves appeared slightly broader and of a brighter green colour than in the controls, but no consistent results were obtained and after a few weeks any such effects disappeared and there was certainly no better development than in cultures without adenine. Growth inhibition was almost complete with 10^{-3} adenine and only a small first leaf appeared. With adenine at 2×10^{-4} growth was slower than in the other cultures and the roots became brown, but development continued.

DISCUSSION

An interesting point emerging from the present research is the continued growth of the two species of *Marsilea* when supplied with solutions containing only mineral salts, with glucose as the sole organic constituent, and maintained either in complete darkness or in light of an intensity insufficient to permit of normal development in the absence of sugar. It has been known since the end of the last century that certain flowering plants can utilize dissolved sugars supplied in the external medium. Earlier work on this subject has been reviewed by Miller (1938), while Lee (1950) has discussed more recent contributions, but as far as the present writer is aware the growth of pteridophytes in darkness on organic media has not been demonstrated previously.

There has, in fact, been little published work on the development of pteridophytes in sterile culture, although Wetmore and Morel (1949, 1949a) have carried out fairly extensive studies which at the time of writing have appeared only in abstract form. From the work of Wetmore and Morel, together with the results of the present research, it would appear that the requirements for the culture of pteridophytes are not appreciably different from those of the flowering plants.

Studies dealing with the role of growth substances in pteridophytes are also comparatively few, but their overall effect is to confirm the belief that these plants show substantially the same responses as the flowering plants. See Williams (1937), Albaum (1938), Seidl (1941), Hurel-Py (1943), Strickler (1946), Pilet (1949), Wetmore and Morel (1949, 1949a), and Wetmore and Pratt (1949). There is little information, however, as to the histological effects of growth substances in the pteridophytes.

Gautheret (1945) has summarized the action of heteroauxins on plant tissues and considers that normal physiological reactions are found only at concentrations of 10^{-7} g./l. or lower, while concentrations of 10^{-5} upwards induce a stage of cellular expansion, which is quite distinct from the cell elongation at lower physiological concentrations, and leads to the production of giant cells and subsequent death of the tissue. Thomson (1945) concluded that cellular proliferation is the most consistent reaction to relatively high concentrations of 'physiologically active substances'.

The response of *Marsilea* to various growth substances, although differing in detail, is in general agreement with the above conclusions. Cellular proliferation of the rhizome tissues was obtained with each substance employed. The reaction of the roots was particularly clear. With both IAA and NAA there was a marked inhibition in rate and amount of root growth at 10^{-6} g./l. An inhibition at such concentrations has been reported frequently (Thimann, 1948). It has been implied by White (1943, p. 188) that the apparent production of many laterals at higher concentrations of IAA is a consequence of the shortening of the root axis producing a crowding of the laterals and thus simulating a rooting response. But such an explanation is certainly not valid in the case of *Marsilea*, where at 10^{-6} IAA certain roots form many laterals, which are completely absent in corresponding roots of cultures grown in media lacking the growth substance. With NAA no such production of laterals was observed, and with neither substance was there a rooting response in the rhizome tissues. The case of 2,4-D calls for special comment. This substance has evidently a remarkable capacity for inducing root formation and at a concentration of 10^{-7} stimulated the origin of supernumerary roots in the parenchyma of the rhizome and even of the leaf bases. At concentrations of 5×10^{-7} g./l. 2,4-D distinct root tumours were formed. Less definite swellings produced in IAA were a result of expansion of the cortical cells in a fashion similar to that already reported by Gautheret (1945) for many plant tissues. Similar root tumours have been described by Carlton (1943) as a response of the roots of certain monocotyledons to relatively high concentrations of NAA and IAA.

There is no accepted explanation of the unusually great activity of 2,4-D and similar substances, but Skoog (1947) has proposed the interesting hypothesis that such substances act by displacing free auxin from the protein centres of inactive auxin-protein complexes. Guttenberg (1942) and Dettweiler (1942) had earlier advanced the view that all synthetic auxins including IAA owe their characteristic activity to the release of the true auxins.

In recent years a number of investigations have indicated that adenine may be an important factor in morphogenesis (Bonner and Haagen-Smit, 1939; Bonner and Bonner, 1940; Thimann and Poutasse, 1941; Skoog and Tsui, 1948; and Galston and Hand, 1949). In the present work, however, no consistently better growth was obtained in cultures of *Marsilea* supplied with a wide range of concentrations of adenine. De Ropp (1945) and Kruyt and

Veldstra (1947) were also unable to confirm the pronounced effects of adenine on leaf growth referred to in earlier work.

Among the results reported in the present contribution certain less direct aspects of the action of the growth substances are also of interest. An example of this kind was provided by the recovery to normal growth of the abnormal plants resulting from the effects of IAA, NAA, and 2,4-D. This recovery presumably occurred after the utilization or destruction of the added growth substance, although this point was not specially investigated. Ball (1944) was impressed by the similar recovery of shoot apices of *Tropaeolum majus* which had been treated with lanolin emulsions of IAA and indolepropionic acid.

Other observations are of interest in relation to the problem of heteroblastic development, a term used by Goebel (1900) to designate the condition in which the juvenile stages show relatively great differences from the adult form. Various views on this subject have been considered by Ashby (1948) in his recent studies on the morphogenesis of leaves.

The successive juvenile forms of the leaves of *Marsilea* have already been described and their nature in ill-nourished cultures has been advanced in support of the view (Goebel, 1900) that the juvenile leaves are arrested formations resulting from inadequate nutrition. The importance of nutrition is suggested also by certain effects of NAA and 2,4-D. Thus, in 10^{-6} NAA, as already described and figured, only simple leaves were produced during a period of strong root inhibition, while the eventual appearance of normal roots was accompanied by a gradual recovery to the normal adult leaf form. In 10^{-7} 2,4-D the first leaf developed normally, but the growth of subsequent leaves was almost completely arrested. As in the case of NAA, the arrest of the leaves is not necessarily a direct effect of the 2,4-D but may be occasioned by the diversion of plastic materials from the developing leaves to the abnormally numerous root primordia. This view is borne out by some of the cultures in 10^{-8} and 5×10^{-8} 2,4-D. In certain sporelings, such as that whose leaves are illustrated in Text-fig. 6, after a phase of normal development leading to the establishment of a typical quadrifid leaf, a sudden appearance of supernumerary roots was accompanied by a reduction in size of the leaf although the quadrifid condition was at first retained. In later leaves some of the leaf lobes were reduced to minute spikes, but with a return to normal root development the typical leaf form was again attained. In this case it seems likely that the leaf abnormalities are due to diversion of materials into the supernumerary roots.

Troll (1939, p. 1389) emphasizes the importance of the shoot apex in heteroblastic development, although he adheres to Goebel's general conception that the juvenile leaves are arrested forms of the later leaves. Troll considers that as the growing-point increases in size so do the leaf primordia and consequently continue their development for a longer period. This view is in keeping with the observations discussed above. It is considered that in the cultures on 10^{-6} NAA, and in media without glucose, the nutrition of the rhizome apex was impaired at an early stage when only simple leaves could develop. In the cultures in 2,4-D which suddenly produced supernumerary

roots, the apex had already attained a development adequate for the initiation of more mature leaves. The diversion of materials into the root primordia apparently prevented the completion of the subsequent development of the leaf primordia but was insufficient to influence the type of primordia produced. This view is supported by the fact that the degree of division of the lamina is determined at a very early stage. This is clearly shown in Pl. XI, Fig. 11, where the lamina of the leaf primordium of the small lateral branch already shows the division into the four lobes characteristic of the expanded leaf.

In the sporelings just discussed the reduction of certain leaf lobes to small spikes is of interest in relation to views as to the nature of the sporocarp. Mahabale and Gorji (1948) observed the occurrence of similar spike leaflets on plants collected from a natural habitat. They considered that these spikes resemble the teeth found on the mature sporocarp of many species of *Marsilea* and supply an additional point in support of the foliar nature of the sporocarp.

In conclusion, the present work has shown that the action of certain representative growth substances on *Marsilea* is similar to that repeatedly recorded in the case of the flowering plants. Certain indirect effects suggest a relationship between leaf morphology and the nutrition of the sporeling. This aspect is under investigation at present and will be considered in a later contribution in the present series.

SUMMARY

A method of growing *Marsilea* in sterile culture is described. Beginning with fertilized 'spores', the sporelings grow well in artificial light, or even in darkness, in liquid media containing glucose as the sole organic constituent. In darkness, etiolated plants are obtained, but in the light, development is similar to that described for natural conditions.

It is shown that addition to the medium of indolacetic acid, α -naphthaleneacetic acid, or 2,4-dichlorophenoxyacetic acid results in responses which vary according to the concentration and nature of the substance supplied. These effects are described and figured. In general, they are similar to those previously reported for other plants. Addition of adenine, guanine, or uracil, however, has no effect over a wide range of concentrations.

When plants are grown under conditions of inadequate nutrition the resulting leaves are much simpler in form than those normally found. These observations are discussed in relation to views on heteroblastic development, and the changes in leaf morphology produced by the addition of the three growth substances are also considered in the same connexion.

I wish to thank Professor C. W. Wardlaw for his encouragement and advice during the progress of this work. I am also grateful to Professor R. H. Wetmore for his kindness in supplying details of his culture methods and results prior to publication, and to Professors Gilbert M. Smith and D. S. van Fleet, and Dr. N. Beadle for gifts of *Marsilea* sporocarps. Thanks are due to Mr. E. Ashby for the majority of the photographic illustrations, and to Mr. P. T. Dawes for Figs. 1-5 of Pl. X.

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DESCRIPTION OF PLATES X AND XI

Illustrating A. Allsopp's article on 'Experimental and Analytical Studies of Pteridophytes, XVII. The Effect of Various Physiologically Active Substances on the Development of *Marsilea* in Sterile Culture'.

FIG. 1. *Marsilea Drummondii* after 4 months of growth on 1 per cent. agar medium containing 3 per cent. glucose. ($\times \frac{3}{8}$.)

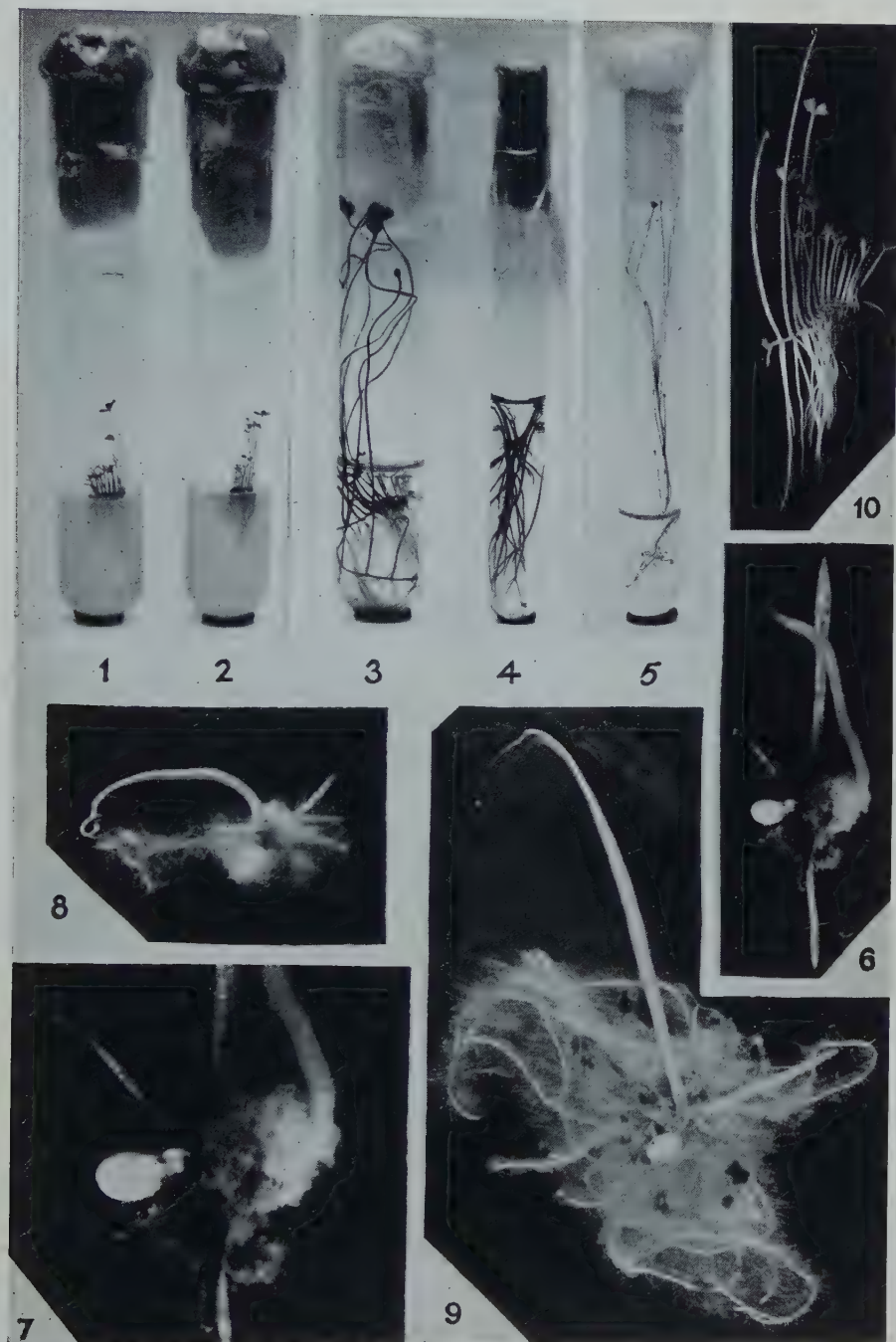
FIG. 2. *M. vestita* after 3 months of growth on the same medium as in Fig. 1. ($\times \frac{3}{8}$.)

FIG. 3. *M. Drummondii* after 3 months of growth in liquid medium containing 3 per cent. glucose. ($\times \frac{3}{8}$.)

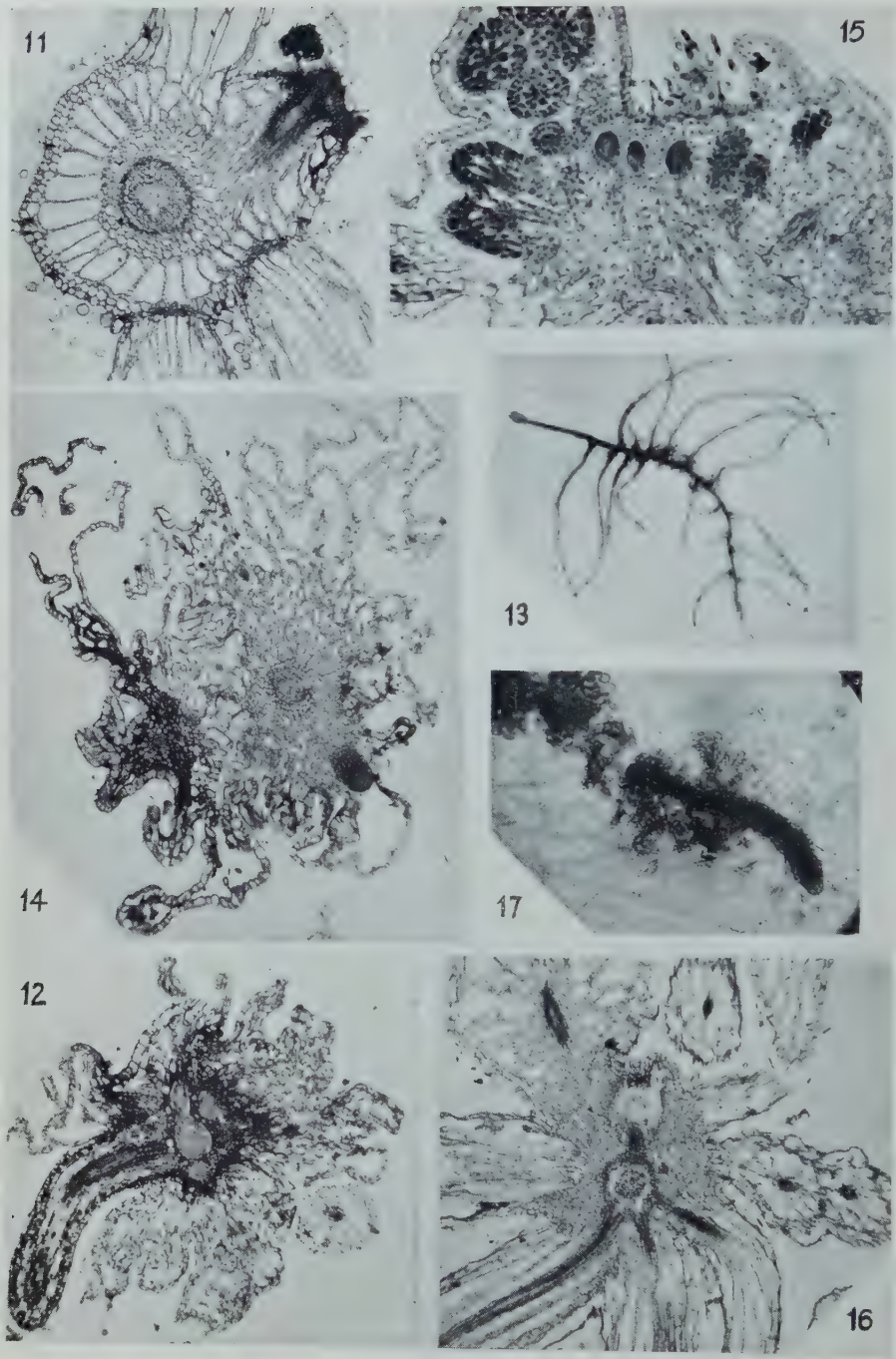
FIG. 4. *M. vestita* after 11 weeks in liquid medium containing 3 per cent. glucose. ($\times \frac{3}{8}$.)

FIGS. 5-10. *M. Drummondii*. Fig. 5. After 4 months in the dark in liquid medium containing 3 per cent. glucose. ($\times \frac{3}{8}$.) Fig. 6. After 6 weeks in medium containing 3 per cent. glucose and 5×10^{-7} NAA. ($\times 6$.) Fig. 7. Enlarged view of rhizome region of sporeling of Fig. 6. ($\times 12$.) Fig. 8. After 2 weeks in medium containing 3 per cent. glucose and 10^{-6} 2,4-D. ($\times 6$.) Fig. 9. After 7 weeks in medium containing 3 per cent. glucose and 5×10^{-7} 2,4-D. ($\times 6$.) Fig. 10. After 10 weeks in medium containing 3 per cent. glucose and 10^{-8} 2,4-D. ($\times 1$.)

FIGS. 11-17. *Marsilea Drummondii*. Fig. 11. Transverse section of a plant grown for 12 weeks in a medium originally containing 3 per cent. glucose and 10^{-6} NAA. The rhizome is now of normal structure. The section shows the bases of two roots and one leaf, and the apical meristem of a lateral branch. ($\times 50$.) Fig. 12. Transverse section of the abnormal earlier part of a plant grown for 14 weeks in a medium containing 3 per cent. glucose and 10^{-5} IAA. Note the callus-like expansion of the cortex. ($\times 50$.) Fig. 13. Root from plant grown for 14 weeks in the dark in a medium containing 3 per cent. glucose and 10^{-6} IAA. ($\times 5 \frac{1}{2}$.) Fig. 14. Transverse section of a plant shown in Pl. X, Figs. 6 and 7. An arrested root primordium is seen to the lower right. ($\times 50$.) Fig. 15. Longitudinal section of the plant illustrated in Text-fig. 5 (b), i.e. after 2 weeks in 10^{-7} 2,4-D. Fasciated roots are seen on left and several root primordia immediately below apical region. ($\times 100$.) Fig. 16. Transverse section of the plant illustrated in Pl. X, Fig. 10, through the region bearing supernumerary roots. Roots arise on all sides of the rhizome in contrast to the usual arrangement seen in Fig. 1. ($\times 50$.) Fig. 17. Apical region of root from a plant grown for 11 weeks in a medium containing 10^{-7} 2,4-D. Loosening of cortical cells and numerous root-hairs are shown. ($\times 55$.)



A. ALLSOPP



A. ALLSOPP

The Carbohydrate Nutrition of Tomato Roots

III. The Effects of External Sucrose Concentration on the Growth and Anatomy of Excised Roots

BY

H. E. STREET AND S. M. MCGREGOR

(Department of Botany, University of Manchester)

With Plates XII and XIII and fourteen Figures in the Text

ABSTRACT

Variation of the concentration of sucrose in White's medium markedly affects the increase in length of the main axis, and the number and total length of the laterals developed by excised tomato roots. Highest values for these three features are obtained with medium containing 1.5 per cent. sucrose. Root material has been analysed anatomically. Root diameter, cortical thickness, number of xylem vessels, and development of the cortical air-space system increase with increase in sucrose concentration. The degree of vacuolation of extra-stelar meristematic cells increases, and protophloem differentiates further from the promeristem with rising sucrose concentrations. The interpretation of these results is discussed.

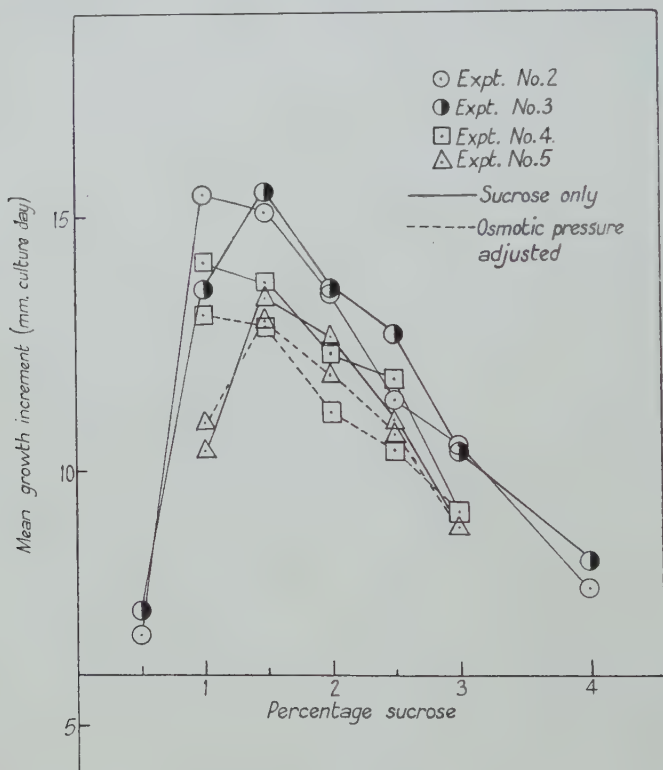
INTRODUCTION

PREVIOUS work (Street and Lowe, 1950) has shown that the growth and development of excised tomato roots were markedly influenced by the concentration of sucrose present in the culture medium. A concentration of 2.0 per cent. sucrose was superior to the other concentrations tested when growth was assessed by measurement of the increase in length of the main axis and by counts of the number of laterals produced. Roots receiving less than 1.0 per cent. sucrose had a shorter and thinner main axis and a reduced number of laterals. Roots supplied with 3.0 or 4.0 per cent. sucrose were also characterized by a shorter main axis but this axis was thick, yellow rather than white, and frequently covered with a dense felt of root-hairs. Laterals were present in normal number per unit length of main axis but showed a reduced rate of elongation. By using raffinose, which has little or no deleterious effect on the growth of roots which are simultaneously supplied with sucrose, it was shown that the effects of media of high sucrose content were not due to their higher osmotic pressures. The present paper gives a more detailed analysis of the effects of the sucrose content of the medium on the growth, morphology, and anatomy of the excised tomato root.

GENERAL EXPERIMENTAL PROCEDURE

The general culture technique has been previously described (Dormer and Street, 1949; Street and Lowe, 1950). A clone of excised tomato roots

(Best-of-All) now in its one-hundred-and-fortieth passage was used. The culture medium (White, 1943) used for maintenance of the stock cultures and as the basis of the experimental media was modified by the inclusion of 0.005 p.p.m. Cu as copper sulphate and 0.001 p.p.m. Mo as molybdic acid (Boll and Street, 1951). All roots were grown at $27^{\circ} \pm 0.5^{\circ}$ C.



TEXT-FIG. 1. The effect of sucrose concentration on the increase in length of the main axis of excised tomato roots. Raffinose (Expt. No. 4) and mannitol (Expt. No. 5) were used in media containing less than 3.0 per cent. sucrose to adjust the osmotic pressure to that of White's medium containing 3.0 per cent. sucrose.

A. The Effects of Sucrose Concentration assessed by Measurement of the Growth of Tip Cultures after 7 days Incubation

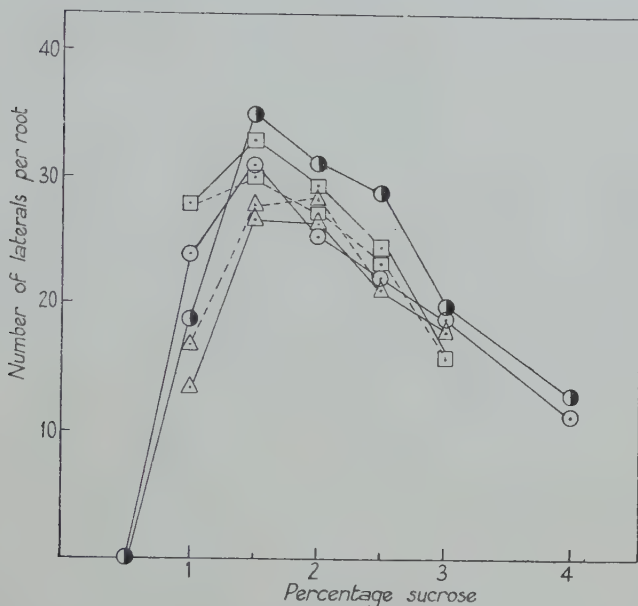
1. Experimental technique

In all experiments growth was assessed by measurement of the increase in length of the main axis (mm./culture/day), by a count of the number of laterals and by measurement of the total length (mm.) of laterals per root. The mean value for each treatment in each experiment is based on examination of 20 replicates. Experiments 1-7 described in this section involved the examination of 980 cultures.

The values for dry weights recorded in Experiments 2, 4, 5, and 6 were obtained by drying the roots for 12-18 hours at 60° C.

2. Results

(a) *The use of raffinose and mannitol to adjust the osmotic pressure.* Experiment 1 (Table 1) showed that mannitol is without deleterious effect on the



TEXT-FIG. 2. The effect of sucrose concentration on the number of laterals developed by excised tomato roots. Key as for Text-fig. 1.

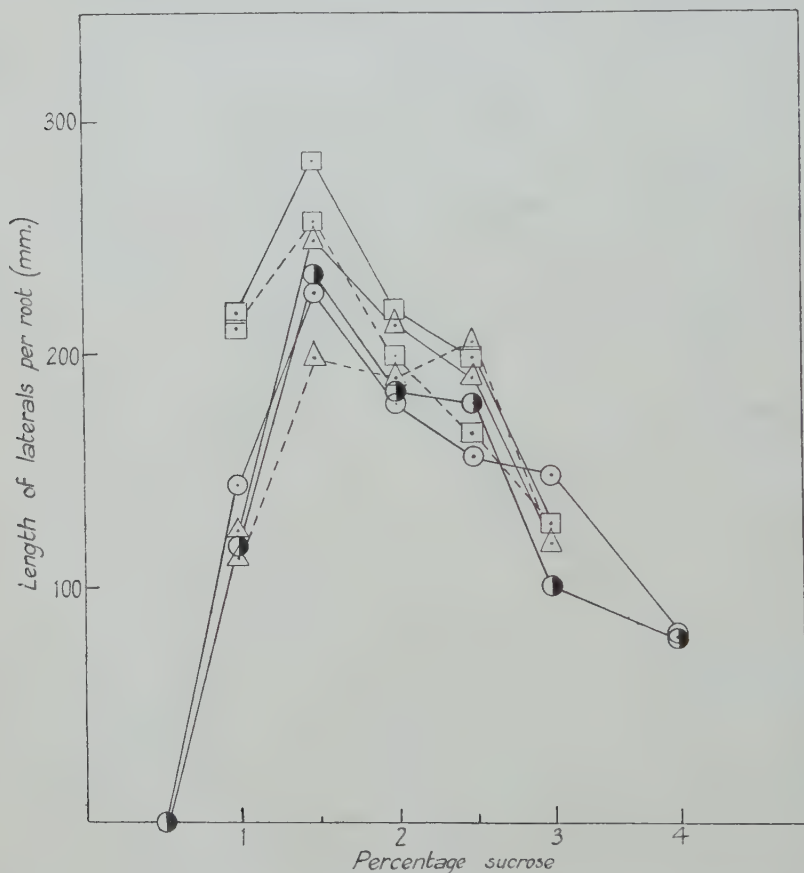
growth of roots simultaneously supplied with sucrose and that it is unable to act as a carbon source for excised roots. In two further experiments the growth of roots in media containing 1.0, 1.5, 2.0, and 2.5 per cent. sucrose

TABLE I

The Growth of Excised Tomato Roots in Media containing Mannitol as the Carbon Source and in Media containing Sucrose and Mannitol (Experiment 1)

Character measured.	Sugar content of the medium.				
	Sucrose 2.0 per cent.	Sucrose 2.0 per cent. + 0.26 per cent. mannitol.	Sucrose 2.0 per cent. + 0.53 per cent. mannitol.	Sucrose 2.0 per cent. + 1.06 per cent. mannitol.	1.06 per cent. mannitol. 2.18 per cent. mannitol.
Increase in length of main axis (mm./culture/day)	11.0	10.5	10.8	11.3	No growth.
No. of laterals per root	23.9	22.4	22.1	22.4	
Length of laterals per root (mm.)	146	158	167	151	

was compared with that in media containing the same sucrose concentrations but adjusted to the osmotic pressure of 3.0 per cent. sucrose medium by addition of raffinose (Experiment 4) and mannitol (Experiment 5). These experiments, using two different adjusting agents, have confirmed that the effects of sucrose concentration here described are not due to changes in the

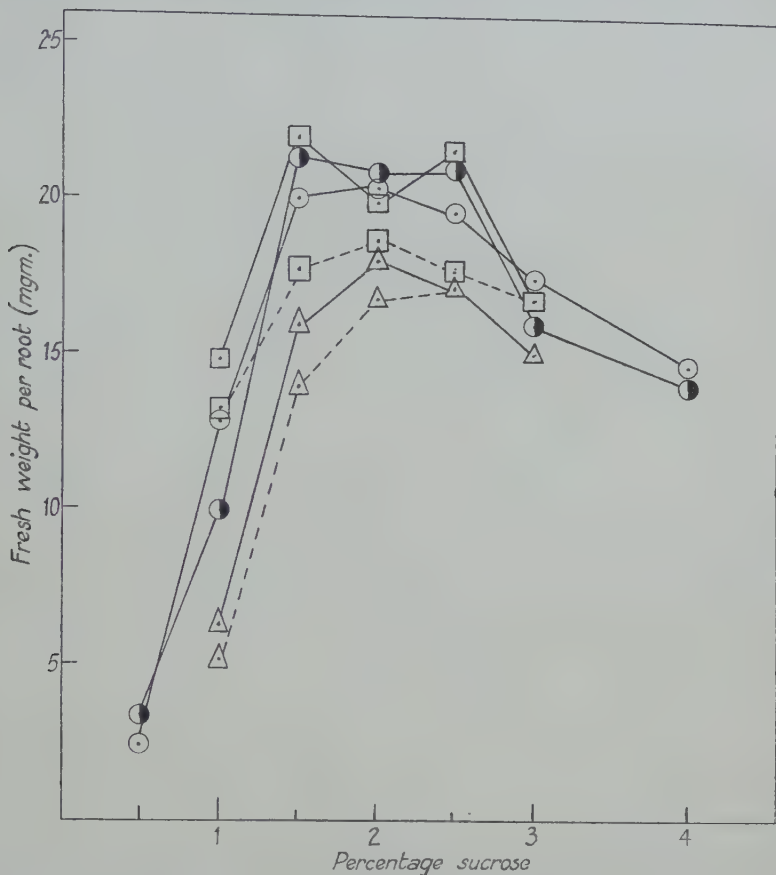


TEXT-FIG. 3. The effect of sucrose concentration on the total length of laterals developed by excised tomato roots. Key as for Text-fig. 1.

osmotic pressure of the medium, although there was in Experiment 4 a reduction in growth in the solutions to which raffinose was added.

(b) *The effect of sucrose concentration on increase in length of the main axis, number of laterals per root, and total length of laterals per root (Text-figs. 1-3).* In this work the highest values for growth in length of the main axis, number of laterals per root, and total length of laterals per root are obtained with 1.5 per cent. sucrose (see Pl. XII). With increase in sucrose concentration above 1.5 per cent. a consistent decrease occurs in all three values. With

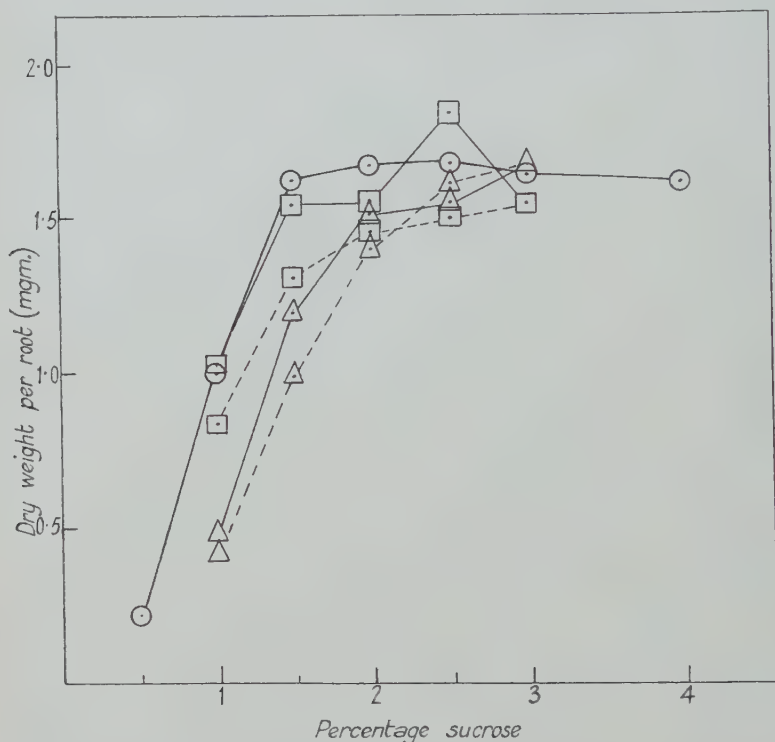
1.0 per cent. sucrose the values obtained for lateral number and total length of laterals are variable and within any one experiment the mean values are below those obtained with 1.5 per cent. sucrose. Increase in length of the main axis with 1.0 per cent. sucrose is equally variable but may equal or even



TEXT-FIG. 4. The effect of sucrose concentration on the fresh weight of excised tomato roots. Key as for Text-fig. 1.

slightly exceed that obtained with 1.5 per cent. sucrose. Such high values for increase in length of the main axis in 1.0 per cent. sucrose have been noted in previous work (Street and Lowe, 1950), and it was thought then that they occurred only when the osmotic pressure was increased by raffinose. It is now clear that such values are also encountered using unadjusted solutions. With 0.5 per cent. sucrose low values are obtained for increase in length of the main axis, and laterals are absent from most roots and never exceed three in number. Judged by these linear growth measurements, 1.5 per cent. sucrose is superior to the other concentrations tested. The general morphology of roots grown in different sucrose concentrations is shown in Pl. XII.

(c) *The effect of sucrose concentration on fresh and dry weights and on percentage dry matter (Text-figs. 4-6).* When growth is assessed on a fresh weight basis (Text-fig. 4) equally high values are obtained with concentrations of 1.5, 2.0, and 2.5 per cent. sucrose. Significantly lower values are obtained with 1.0 and 3.0 per cent. sucrose concentrations and further decreases in fresh weight occur with 0.5 and 4.0 per cent. sucrose.



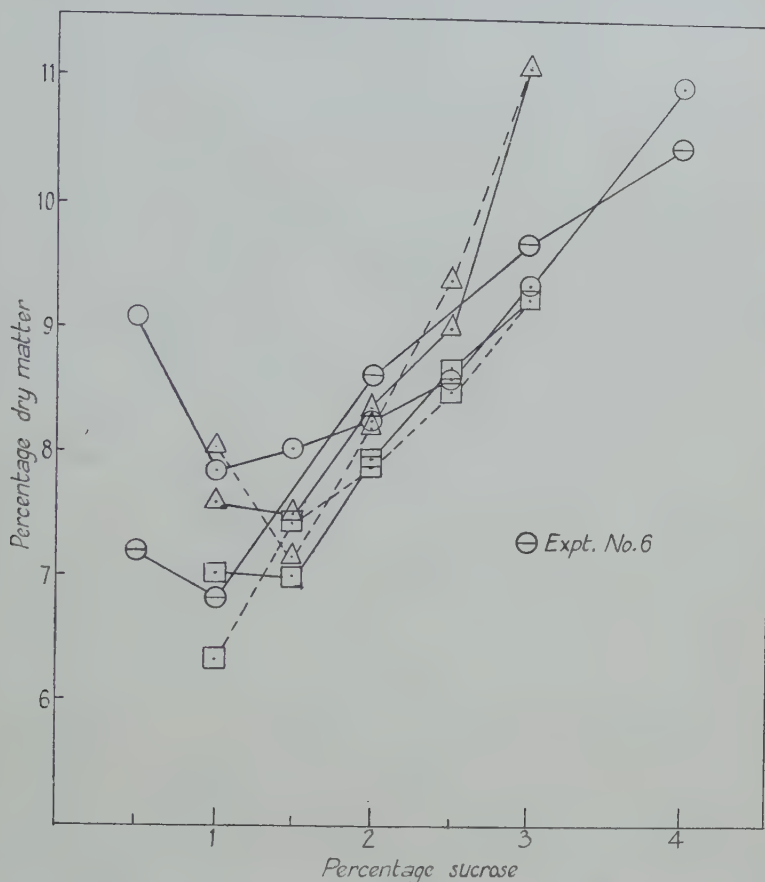
TEXT-FIG. 5. The effect of sucrose concentration on the dry weight of excised tomato roots. Key as for Text-fig. 1.

When growth is assessed on a dry weight basis (Text-fig. 5) uniformly high values are obtained with sucrose concentrations ranging from 2.0 to 4.0 per cent. Below 2.0 per cent. there is a decrease in dry weight with decrease in sucrose concentration.

High values for dry weight from 2.0 to 4.0 per cent. sucrose, although the fresh weight decreases at concentrations above 2.5 per cent., imply that the percentage dry matter increases over the range 1.5 to 4.0 per cent. of sucrose (Text-fig. 6). The percentage dry matter does not diminish further below 1.5 per cent. sucrose, probably owing to the marked decrease in lateral root formation at low sucrose concentrations. Thus at 0.5 per cent. sucrose the percentage dry matter of a main axis devoid of laterals is being measured.

B. *Growth Curves of Roots supplied with 1.5 and 3.0 per cent. sucrose (Text-fig. 7)*
Experimental technique

One hundred and twenty cultures in 1.5 per cent. and 120 cultures in 3.0 per cent. sucrose were set up on the same occasion (Experiment 8) and a

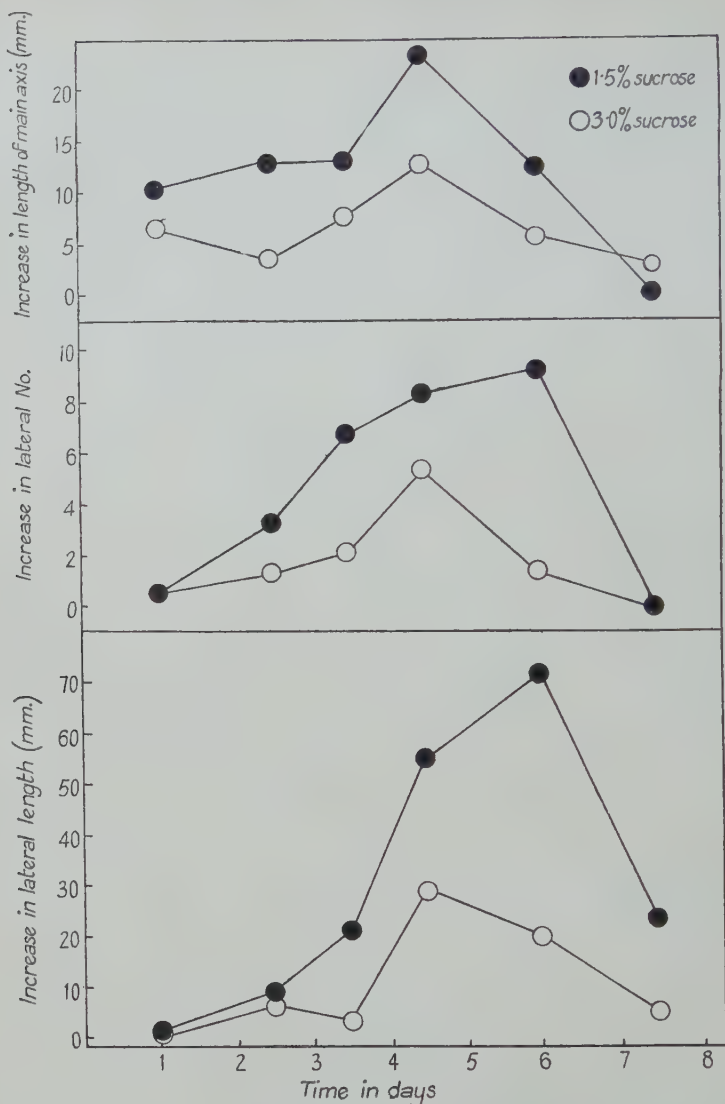


TEXT-FIG. 6. The effect of sucrose concentration on the percentage dry matter of excised tomato roots. Key as for Text-fig. 1.

random sample of 15 roots harvested from each sucrose concentration at intervals during a growth period of 8 days. At each sampling, measurements were made of length of main axis, lateral number, and total length of laterals per root. The mean increase in these measurements per 24 hours was calculated at each sampling time and plotted at the midpoint of the time interval (Text-fig. 7).

Results

The growth curves for both sucrose concentrations show a marked similarity, with a clearly defined peak for growth in length of the main axis,



TEXT-FIG. 7. The mean daily growth increment curves for increase in length of main axis, in number of laterals, and in total length of laterals of excised tomato roots grown in media containing 1.5 and 3.0 per cent. sucrose (Expt. No. 8).

between 4 and 5 days after inoculation. The temporary check in growth of the main axis which in some previous experiments (Street and Lowe, 1950) has been observed to precede the peak value, occurred in the present experiment between the third and fourth days with 1.5 per cent. sucrose and between the second and third days with 3.0 per cent. sucrose. This was accompanied by a shift of the peak value for increase in lateral number and in lateral length to between the fifth and seventh days in 1.5 per cent. sucrose as compared with

between the fourth and fifth days for 3.0 per cent. sucrose. The higher values for linear growth in 1.5 per cent. sucrose were primarily due to an increased rate of growth at this concentration rather than to any earlier diminution of growth rate in 3.0 per cent. sucrose.

C. *The Anatomy of Excised Roots grown in Various Sucrose Concentrations*

1. *Experimental techniques*

Root material obtained from 7-day-old tip cultures was fixed, sectioned at a thickness of 6 microns, and stained by Sharman's method (Sharman, 1943).

(a) *Longitudinal and transverse sections of the root tip.* The tips were treated with Navashin's fixative (McLean and Cook, 1941) for 24 hours and longitudinal and transverse sections prepared. Measurements of root diameter, stele diameter, cortical thickness, and number of cortical cell layers were made on median longitudinal sections at the level at which the cells of the piliferous layer begin to elongate. The cortex was taken to include the endodermis and exodermis but not the piliferous layer.

(b) *Transverse sections at 17–20 mm. from the root apex.* The roots were fixed in formalin-aceto-alcohol for 24 hours. A ribbon of not less than twenty-five sections was prepared from each root and measurements were made on three sections: one at each end and one in the middle of the ribbon. The values for root and stele diameters are the means of two measurements, one along the diameter passing through the protoxylem groups of the diarch xylem, and the other along the diameter at right angles to this. The cortical thickness and number of cortical cell layers are each means of values derived from either side of the stele along the diameter through the protoxylem groups. The numbers of cells in the endodermis and piliferous layer were calculated from counts in the half circumference as indicated by the two protoxylem poles. Values were also obtained for xylem width in microns and in number of xylem cells and also for the number of lignified xylem vessels placed laterally to the single row along the diameter passing through the protoxylem groups.

(c) *Preparations of whole roots.* Roots fixed in 70 per cent. alcohol were cleared with lactophenol and used for measurement of the length and width of cells of the piliferous layer and of the exodermis at 15–20 mm. from the root apex. The mean values obtained for each root for length and width of cells of the piliferous layer and exodermis were based on ten separate measurements. Determinations were also made of the distance from the promeristem to the occurrence of spirally thickened protoxylem vessels in both xylem archs.

Roots, harvested after 4 days' growth in Experiment 8, were fixed in formalin-aceto-alcohol, cleared in lactophenol, and used to measure the length of piliferous layer cells at selected distances from the promeristem. At each distance the length of ten cells was determined on each of the five roots examined per sucrose concentration.

Measurements of root diameter, 20–25 mm. from the root apex, were obtained by examination under the low power of the microscope of the intact fresh roots harvested in Experiments 2–7 (Part A).

2. Results

The results are shown in Text-figs. 8–14 and in Table 2. The values obtained are shown $\pm$ their standard errors. Except where indicated, the number in brackets gives the number of roots examined.

TABLE 2

Quantitative Anatomical Values for Excised Tomato Roots grown in Media containing Different Concentrations of Sucrose

Material.	Character and unit of measurement.	Percentage sucrose.				
		0.5	1.0	2.0	3.0	4.0
Transverse sections 17–20 mm. from root apex.	No. of lignified xylem vessels placed laterally to the single row of cells along the diameter passing through the two protoxylem groups.	1.04 ± 0.21 (12)	0.96 ± 0.39 (11)	1.03 ± 0.30 (12)	3.08 ± 0.64 (11)	5.31 ± 0.88 (12)
„	No. of cells in the piliferous layer.	56.4 ± 2.6 (15)	68.1 ± 2.9 (14)	77.2 ± 2.2 (16)	77.4 ± 2.1 (14)	74.8 ± 3.0 (15)
„	No. of cell layers in the cortex.	4.20 ± 0.12 (19)	4.73 ± 0.15 (20)	5.53 ± 0.22 (20)	6.12 ± 0.19 (20)	6.51 ± 0.29 (20)
Longitudinal sections of root tips.	Distance from promeristem of origin of protophloem (microns).	54.4 ± 1.1 (6)*	58.3 ± 2.4 (7)*	61.8 ± 2.3 (4)*	66.4 ± 5.0 (6)*	86.4 ± 3.6 (10)*
Whole roots lactophenol.	Surface area per cell of the piliferous layer (microns ²).	1,219	1,254	1,226	1,105	1,100
	Surface area per cell of the exodermis (microns ²)	1,916	2,243	2,155	2,258	1,915

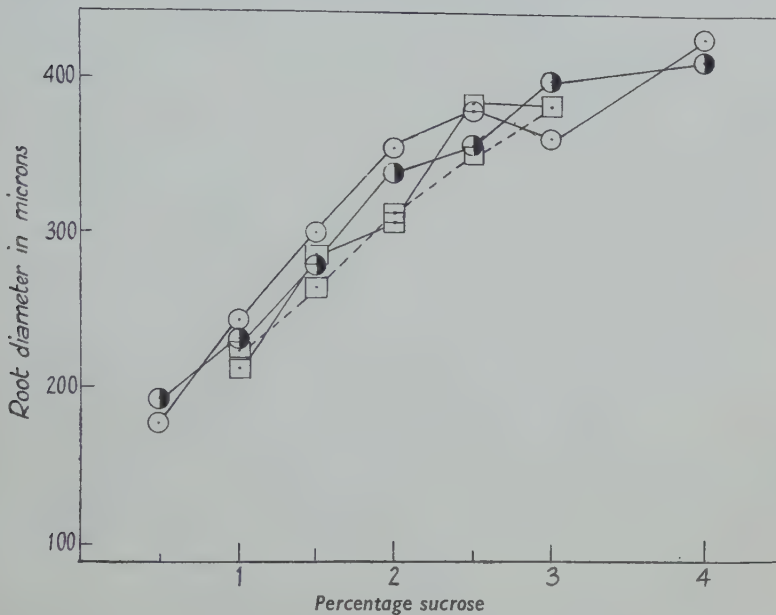
* These replicate numbers represent the number of values recorded and not the number of roots examined.

There is an increase in stelar diameter of roots from 0.5 and 1.0 per cent. sucrose, when sections are cut 17–20 mm. from the root apex. This is associated with a significant increase in xylem width in terms of number of cells (8.07 ± 0.29 (16) and 9.16 ± 0.28 (16) respectively) and in the number of cells in the endodermis (17.6 ± 0.4 (15) and 20.2 ± 0.5 (15)). An increase in sugar concentration above 1.0 per cent. does not lead to any further significant increase in these characters. Increase in concentration of sucrose above 2.0 per cent., however, leads to a significant increase in the number of lignified xylem vessels placed laterally to the single row along the diameter passing through the two protoxylem groups (Table 2 and Pl. XIII).

The increase in cortical thickness is significant between 1.0 and 2.0 and between 2.0 and 3.0 per cent. sucrose in the material sectioned 17–20 mm. from the root apex and between 0.5 and 1.0 and 2.0 and 4.0 per cent. sucrose in the values obtained from longitudinal sections of the root tips (Text-fig. 11). There are significant increases in the number of cell layers in the cortex between 0.5 and 1.0, 1.0 and 2.0, and 2.0 and 3.0 per cent. sucrose (Table 2).

The increase in cortical thickness is associated with an increase in the number of piliferous layer cells between 0.5 and 1.0 per cent. sucrose, but at higher concentrations no further significant increase in this value occurs (Table 2).

Examination of transverse sections, both those cut 17–20 mm. from the root apex and at 160–80 microns from the promeristem, shows that the air-space system of the cortex becomes more highly developed with increase in sucrose concentration up to 2.0 per cent., and that this is associated with an increase

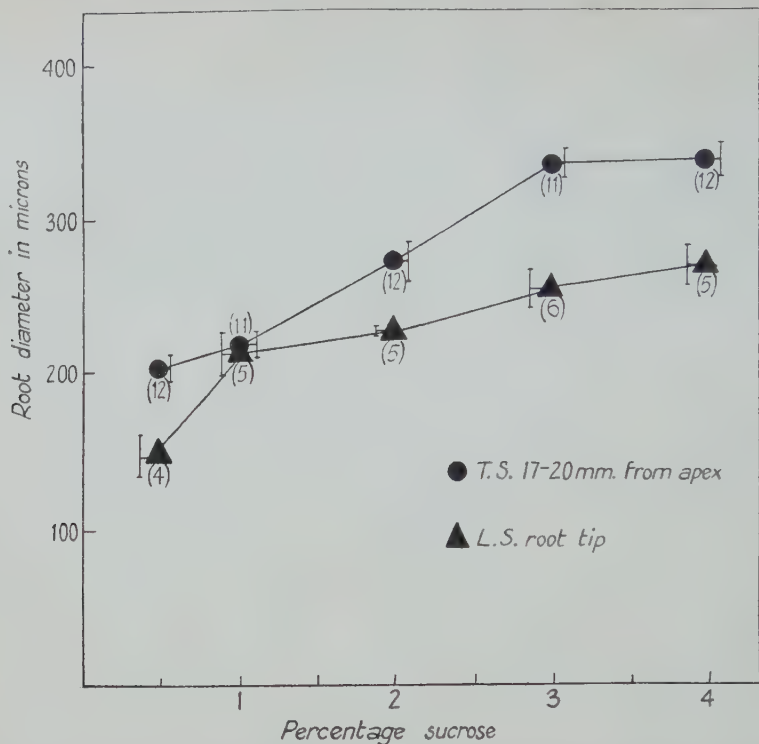


TEXT-FIG. 8. The effect of sucrose concentration on the diameter of excised tomato roots (measurements made on whole fresh roots). Key as for Text-fig. 1.

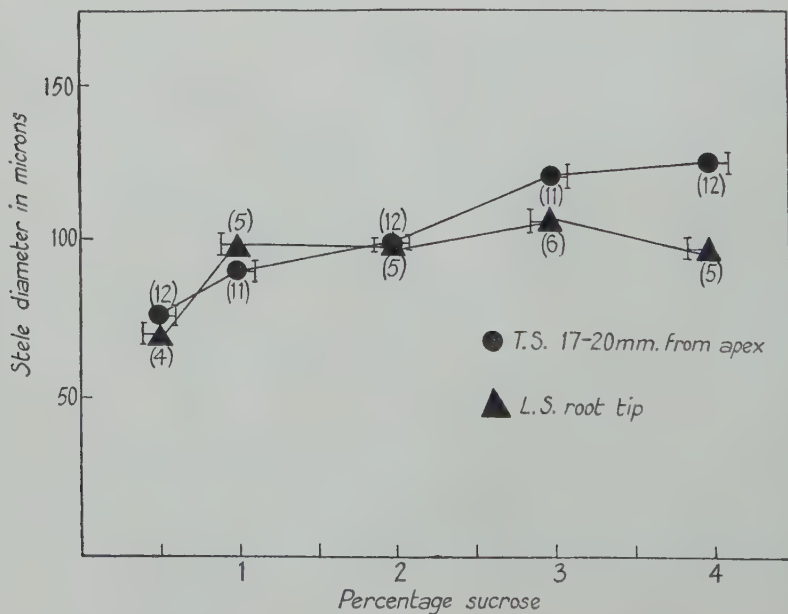
in the area of the cortical cells as seen in transverse section (Pl. XIII). These features, together with the increase in the number of cell layers, account for the increase in cortical thickness with increasing sucrose concentrations.

The measurements of root diameter made on whole roots (Text-fig. 8) and on transverse sections cut 17–20 mm. from the root apex (Text-fig. 9) both show a regular increase in root diameter with increasing sucrose concentration over the range 0.5–3.0 per cent. Burström (1942) has pointed out that the extent of root-hair development may influence the values for root diameter obtained by examination of whole roots. The agreement of the results obtained here by two different methods, however, answers this criticism.

The lack of correspondence between linear growth and increase in weight noted in Experiments 2–6 is in part due to the increase in root diameter with increase in sucrose concentration. Measurements of root diameter made on the longitudinal sections of the root tip (Text-fig. 9) show a significant increase between 0.5 and 1.0 per cent. sucrose but at concentrations above 1.0 per

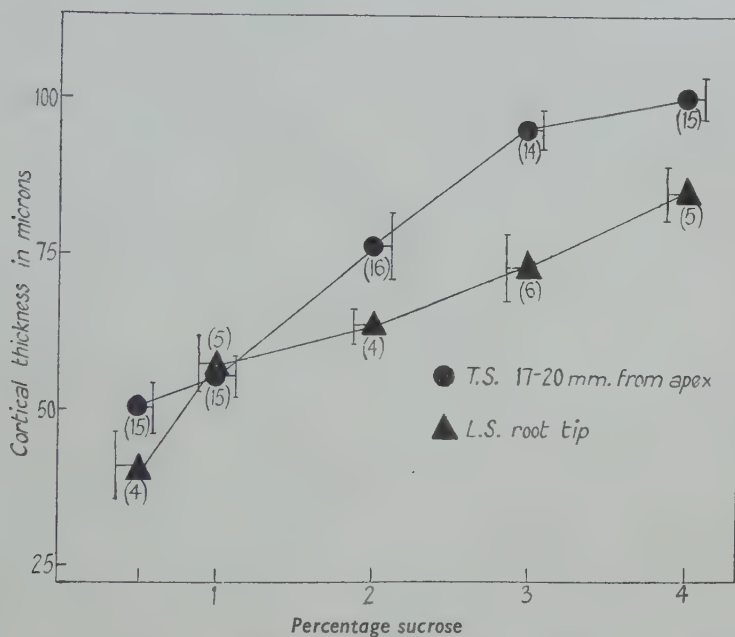


TEXT-FIG. 9. The effect of sucrose concentration on the diameter of excised tomato roots (measurements made on transverse and longitudinal sections). The lengths of the vertical black lines represent twice the standard errors of the mean values for diameter plotted with the mean as midpoint. The numbers in brackets represent the number of roots measured in each treatment.



TEXT-FIG. 10. The effect of sucrose concentration on the diameter of the stele of excised tomato roots. Standard errors and the number of roots examined are indicated as in Text-fig. 9.

cent. sucrose the increase in diameter is less marked. This may be related to the fact that the main contrast between roots grown in 0.5 and those grown in 1.0 per cent. sucrose is the increase in stele diameter (Text-fig. 10). Cortical thickness which principally accounts for the increase in root diameter with increase in sucrose concentration between 1.0 and 4.0 per cent. (Text-fig. 11) has not reached its full value at the point where measurements were



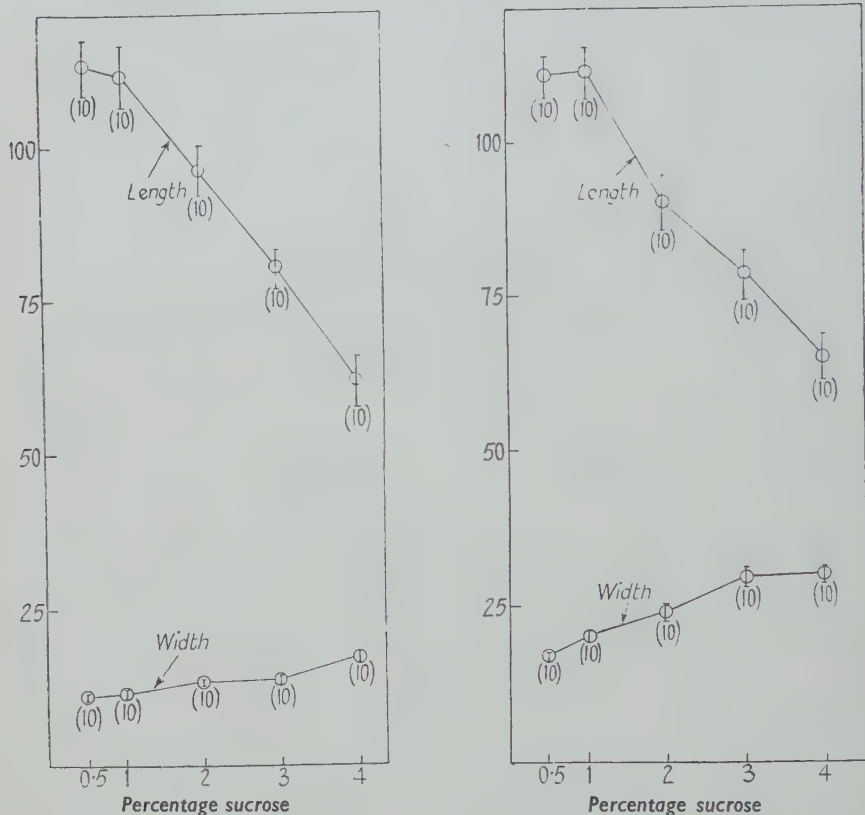
TEXT-FIG. 11. The effect of sucrose concentration on the thickness of the cortex of excised tomato roots. The standard errors and number of roots examined are indicated as in Text-fig. 9.

made on the longitudinal sections. This is also illustrated by transverse sections cut 160–80 microns from the promeristem (Pl. XIII).

Examination of whole roots cleared in lactophenol shows that the tangential width of both piliferous layer and exodermal cells increases with increase in sucrose concentration over the complete range of concentrations tested, and that this is associated with a decrease in the length of the cells in higher concentrations. Mean values for the area per cell of the outer wall in those layers were calculated by multiplying mean cell length by mean cell width for each sucrose concentration (Text-figs. 12 and 13), and are shown in Table 2. It is clear that these values remain constant over the range of sucrose concentrations tested.

Determination of the lengths of piliferous layer cells at selected distances from the promeristem, using roots harvested in Experiment 8 on the fourth

day of culture, enabled us to plot curves showing the progress of cell elongation in 1.5 and 3.0 per cent. sucrose (Text-fig. 14). With 1.5 per cent. sucrose cell elongation is complete 2.3 mm. from the promeristem, and with 3.0 per cent. sucrose 1.7 mm. from the promeristem. The time-scales given were



TEXT-FIGS. 12-13. The effect of sucrose concentration on the dimensions of cells of the piliferous layer (on left) and of the exodermis (on right), determined by examination of whole roots cleared in lactophenol. Standard errors and the number of roots examined are indicated as in Text-fig. 9.

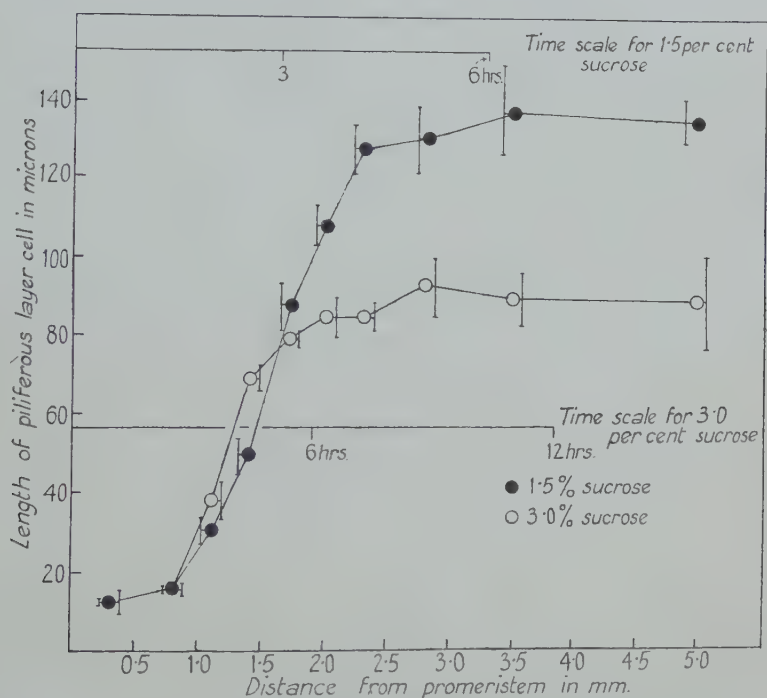
calculated from the mean increases in length of the main axis during the previous 24 hours in culture. The marked difference in linear growth rate in the two sucrose concentrations makes possible an approximate estimate of the duration of the phase of elongation for each treatment. The greater length of the piliferous layer cells in 1.5 as compared with 3.0 per cent. sucrose is seen to be due to a much more rapid rate of elongation proceeding for a slightly shorter time.

With 0.5 and 1.0 per cent. sucrose, sections cut 17-20 mm. from the root apex show a typical primary endodermis with Casparian strips, but with 3.0 and 4.0 per cent. sucrose the endodermal cells are uniformly thickened and

frequently contained a yellow deposit most readily seen before staining. This deposit accounts for the yellowing of the roots observed in the higher sucrose concentrations. At these concentrations there is also an increase in the thickness of the walls of the cells of the exodermis.

Root tips. Examination of the sections of root tips showed that:

(i) With 3.0 and 4.0 per cent. sucrose almost all the extra-stelar cells of the meristem are highly vacuolated (Pl. XIII). With concentrations ranging from



TEXT-FIG. 14. Curves showing the course of elongation of piliferous layer cells of 4-day-old excised tomato roots grown in media containing 1.5 and 3.0 per cent. sucrose. Measurements were made at selected distances from the promeristem of whole roots cleared in lactophenol. The time-scales shown have been calculated from the mean increase in length of the roots during the previous 24-hour period (Expt. No. 8). Standard errors are indicated as in Text-fig. 9.

0.5 to 2.0 per cent. sucrose the number of vacuolated cells is smaller and vacuolation can be seen to start first in the inner cortex and then to spread gradually outwards (Pl. XIII). The endodermal and particularly the piliferous layer cells are both late in vacuolating. The extent of vacuolation was least in 0.5 per cent. sucrose and seemed to increase with each increase in sucrose concentration, though this was not studied quantitatively.

(ii) Two single files of protoxylem cells occur between the two protoxylem groups and immediately adjoin the pericycle. They can first be identified by

their cytoplasm staining blue in contrast to the pink colour of the cytoplasm of the surrounding cells of the stele. As differentiation proceeds the cells vacuolate and their walls, particularly their transverse walls, stain blue-black. The characteristic staining reaction of these protophloem cells makes possible an estimate of the distance between the promeristem and the first appearance of differentiating phloem where such cells are observable in median or near-median longitudinal sections. In the region of the promeristem a line is distinguishable, demarcating the root-cap from those cells which give rise to the stele and cortex. Measurement of the distance between this line and the origin of the protophloem has been possible in a limited number of sections, and the mean values obtained are shown in Table 2. Adjoining sections were also examined to ensure that the measurement was made on the section including the youngest protophloem. Where protophloem was distinguishable on both sides of the stele, two values were obtained, and in this case the replicate number quoted is the total number of values recorded and not the number of roots examined. There is evidence that protophloem differentiation occurs farther from the promeristem in high sucrose concentrations than in low. Similar observations were made on the origin of protoxylem vessels using whole roots cleared in lactophenol, but no correlation could be established between the point of origin of protoxylem and sucrose concentration.

DISCUSSION

The growth and anatomy of the excised tomato root is markedly affected by the sucrose concentration of the culture medium. At 0.5 per cent. sucrose the growth is limited by sucrose availability. Burström (1941), in an anatomical investigation of the epidermis of wheat roots grown from seedling tips for 9–15 days in media containing various concentrations of dextrose, concluded that at concentrations below 0.18 per cent. the sugar acted as a limiting nutritive factor. At such low dextrose concentrations the number of cell divisions in the dermatogen was considered to be depressed. Burström quotes values for 'division rates' of the order of 1.3×10^3 divisions per day at 0.018 per cent. dextrose rising to $2.1\text{--}2.3 \times 10^3$ for 0.18 per cent. and then remaining constant up to 1.8 per cent., which was the highest concentration tested. Table 3 shows values for the increase in the number of piliferous layer cells per day calculated from our data, using for each sucrose concentration average values

TABLE 3

Sucrose concentration per cent.	Calculated value for increase in the number of piliferous layer cells per day.
0.5	1.5×10^3
1.0	8.5×10^3
2.0	9.5×10^3
3.0	8.7×10^3
4.0	6.1×10^3

for the increase in length of the main axis, length of piliferous layer cells, and the number of piliferous layer cells as seen in transverse sections. These approximate values illustrate the high 'division rate' occurring in excised tomato roots cultured in White's medium, the depressing effect of the low sucrose concentration and the constancy of the value over the range 1.0 to 3.0 per cent. sucrose.

The similarity observed between the forms of the daily growth curves obtained with 1.5 and with 3.0 per cent. sucrose (Text-fig. 7) might have been expected, since over the range 1.0 to 3.0 per cent. there is over a 7-day culture period close agreement in the total number of new piliferous layer cells formed. It is not possible from our data, or from that presented by Burström, to determine the component factors controlling 'division rates' calculated in this way. Such 'division rates' may depend upon the number of meristematic cells generating the piliferous layer, their division rate, or the number of divisions taking place outside the meristematic region, or a combination of these factors.

The roots grown in 0.5 per cent. sucrose are characterized by a slow rate of increase in length, an absence of laterals, and in giving lowest values for root and stele diameters, cortical thickness, number of cell layers in the cortex, and number of cells in the piliferous layer. The significant increase in stele diameter between 0.5 and 1.0 per cent. sucrose is associated with an increase in xylem width expressed in terms of number of cells and in the number of cells in the endodermis. Above 1.0 per cent. sucrose no further significant increase in either character or in stele diameter occurs.

Use of 1.0 per cent. sucrose results in a more variable growth rate than any other concentration tested (Text-figs. 1-5). Within one experiment some of the roots may resemble those receiving 0.5 per cent. sucrose, and some those obtained with 1.5 and 2.0 per cent. sucrose. In their anatomical characters these roots are in most features between those receiving 0.5 per cent. and those receiving 2.0 per cent. sucrose, but resemble those receiving 0.5 per cent. in the uniform primary condition of the endodermis and in the low value for cortical thickness. At 1.0 per cent. we have a critical sugar concentration at or immediately below which carbohydrate deficiency symptoms appear and immediately above which we obtain the high and uniform rate of linear growth characteristic of roots supplied with 1.5 per cent. sucrose.

With 3.0 per cent., and particularly with 4.0 per cent. sucrose, the roots show a marked decrease in their linear growth rate and in the number of lateral roots developed. The main axis becomes thick, yellow, and brittle and is frequently distorted. These changes are associated with a high percentage dry matter (Text-fig. 6), with the development of additional lignified xylem vessels (Table 2) and of markedly thickened cell walls in both the endodermis and exodermis. The extra-stelar cells of the apical meristem become vacuolated down to the region of the promeristem (Pl. XIII). This vacuolation gives the cells a characteristic appearance and possibly represents an abnormal condition which may either be a direct effect of high sucrose concentration

or it may be related to the very slow rate of increase in length by the end of the seventh day when roots are in media of high sugar content.

Almestrand (1949) noted that cessation of cell division at the root apex occurred when attempts were made to obtain excised barley or oat roots in culture. His examination of whole root apices under low magnification showed that meristems in which cell division had ceased could be identified by the presence of vacuolated cortical cells and of a clearly defined stele in the immediate vicinity of the promeristem. An abnormally high internal level of soluble carbohydrate might be expected to disturb growth and development in just those features which distinguish roots grown in high from those grown in low sucrose concentrations.

Cormack (1947), in a study of epidermal cell development in tomato, white mustard, and maize seedling roots, has described the arrangement of cells and development of air spaces in the cortex of these species. Cormack's preparations show the root cortex of tomato to be built up of cells arranged in compact alternate rows and the air spaces to be small, entirely confined to the inner cortex, and only just discernible at 280–300 microns from the root apex. The arrangement of cortical cells and the early development of large, diamond-shaped air spaces in our excised roots resemble more the conditions described by Cormack as typical of maize roots. This indicates either that the cell arrangement and type of air-space development varies with the tomato variety or that the cortex is profoundly altered when the roots are grown in culture from excised tips.

Goodwin and Stepka (1945) have suggested, in conformity with the views of Priestley (1928), that the activity of the apical meristem of the root may under normal conditions be limited by shortage of food supplies and that the most acute shortage may occur at the root apex. In this way they explained why in roots of *Phleum pratense* the region of most rapid cell division and of root elongation was located not at the apex itself but in a zone 300 to 425 microns back. This zone was traversed by mature sieve-tubes which terminated 230 microns from the apex. If the development of sieve-tubes is controlled by nutrient gradients from the mature cells towards the meristem then it might be expected that phloem differentiation in excised roots would be evident nearest to the apex in roots supplied with low sucrose concentrations and would be delayed when a luxury supply bathed the meristem. In xylem development, the effect of high sugar concentrations takes the form of an increase in the number of lignified vessels (Table 2), in the development of pitted rather than reticulate vessels, and in causing these to occur almost right down to the level at which spiral protoxylem vessels are first identifiable.

Burström (1941) has suggested that, with increase in sugar concentration over the range where 'division rate' is unaffected, the changes in linear growth and root diameter arise from the effect of sugar concentration on the orientation of the plane of cell division, increasing sugar concentration causing a change from division by transverse to division by longitudinal walls. Such an explanation could account for the increase in number of cell layers in the cortex

(Table 2, Pl. XIII) observed to occur in our roots with increase in sucrose concentration. This would imply some change from the normal polarity of the cells from which these tissues are derived, an effect which in its turn could be caused by the removal of normal nutrient gradients by increased sucrose concentration. The fact that there is no significant increase in the number of cells in the piliferous layer (Table 2) with increase in sucrose concentration above 1.0 per cent. does, however, argue against such an explanation.

Increase in sucrose concentration caused the cells of the piliferous layer (Text-fig. 12) and of the exodermis (Text-fig. 13) to change in shape, their length decreasing and their width (tangential) increasing with increase in sucrose concentration. These results are in contrast to the observations of Burström (1941), who found in the epidermis of the wheat root a gradual increase in cell length with increase in dextrose concentration. Burström obtained evidence that the increase in cell length resulted from a more rapid rate of elongation for a decreasing time with dextrose concentrations up to 0.18 per cent. but that above this concentration the rate of elongation did not increase but continued for a longer time. The curves depicting the course of cell elongation in our roots (Text-fig. 14) closely resemble in form those figured by Goodwin and Stepka (1945) for the roots of *Phleum pratense* and indicate that the greater cell length achieved at the lower sucrose concentration is due to a more rapid rate of elongation. It is clear, therefore, that wheat and tomato roots differ in the effect of sugar concentration on cell elongation.

From previous sugar analyses (Dormer and Street, 1949) on White's medium supporting the growth of excised tomato roots, it is obvious that even at the lowest sucrose concentration tested the total amount of sucrose in 50 ml. medium would be more than adequate to support the maximum growth achieved in any concentration. The effects of low sucrose concentration, therefore, do not arise from exhaustion of the medium. Similarly, from previous work (Street and Lowe, 1950) and from the use of raffinose and mannitol as osmotic pressure regulators in the present series of experiments, it is clear that the effects of sucrose concentration are not due to the osmotic pressure of the external culture medium. Sucrose concentration, therefore, probably causes changes in the growth and development by determining sucrose uptake. Further understanding of the effects described in this paper may follow from studies, now being undertaken, of the influence of sucrose concentration on absorption from the external medium and on the respiration and carbohydrate composition of the roots.

SUMMARY

1. Variation of the concentration of sucrose in White's medium markedly affects the increase in length of the main axis, and the number and total length of the laterals developed by excised tomato roots. Highest values for these three features are obtained with medium containing 1.5 per cent. sucrose.

2. Assessed by fresh weight, maximum growth of excised tomato roots is obtained in media containing from 1.5 to 2.5 per cent. sucrose. Assessed by dry weight, maximum growth occurs over the range 2.0–4.0 per cent. sucrose. There is an increase in the percentage dry matter of the roots with increase in sucrose concentration from 1.5 to 4.0 per cent.

3. The effects on the roots of varying the sucrose concentration are not due to resulting changes in the osmotic pressure of the medium.

4. The growth curves of roots supplied with 1.5 and 3.0 per cent. sucrose are similar in form. Evidence is presented that over the range 1.0–3.0 per cent. sucrose, the total number of new piliferous layer cells produced during the 7-day culture period is constant.

5. Root diameter, cortical thickness, number of xylem vessels, and development of the cortical air-space system increase with increase in sucrose concentration.

6. Piliferous layer cells decrease in length and increase in width with rise in sucrose concentration, the area per cell of the outer wall remaining constant. In 1.5 per cent. sucrose their rate of elongation is higher than in 3.0 per cent. and proceeds for a shorter time.

7. The degree of vacuolation of extra-stelar meristematic cells increases, and protophloem differentiates farther from the promeristem with rising sucrose concentrations. It is suggested that this differentiation of the protophloem at greater distances from the promeristem in higher sucrose concentrations may be due to disturbance of normal nutrient gradients.

8. The view is advanced that the sucrose concentration of the medium influences the growth and development of the root by determining the sucrose uptake.

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to Mr. E. Ashby for the photographs in Plates XII and XIII, and to Miss J. Gannon and Mr. E. Roberts for technical assistance. Grateful acknowledgement is also made to the Agricultural Research Council for a grant which has enabled one of us (S. M. McGregor) to take part in this work.

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EXPLANATION OF PLATES

Illustrating H. E. Street and S. M. McGregor's paper on 'The Carbohydrate Nutrition of Tomato Roots'.

PLATE XII

All natural size

Seven-day-old roots cultured at $27 \pm 0.5^\circ$ C. from 10-mm. root tips in White's medium containing 0.5 per cent. (Fig. 1), 1.0 per cent. (Fig. 2), 1.5 per cent. (Fig. 3), 2.0 per cent. (Fig. 4), 3.0 per cent. (Fig. 5), and 4.0 per cent. (Fig. 6) sucrose.

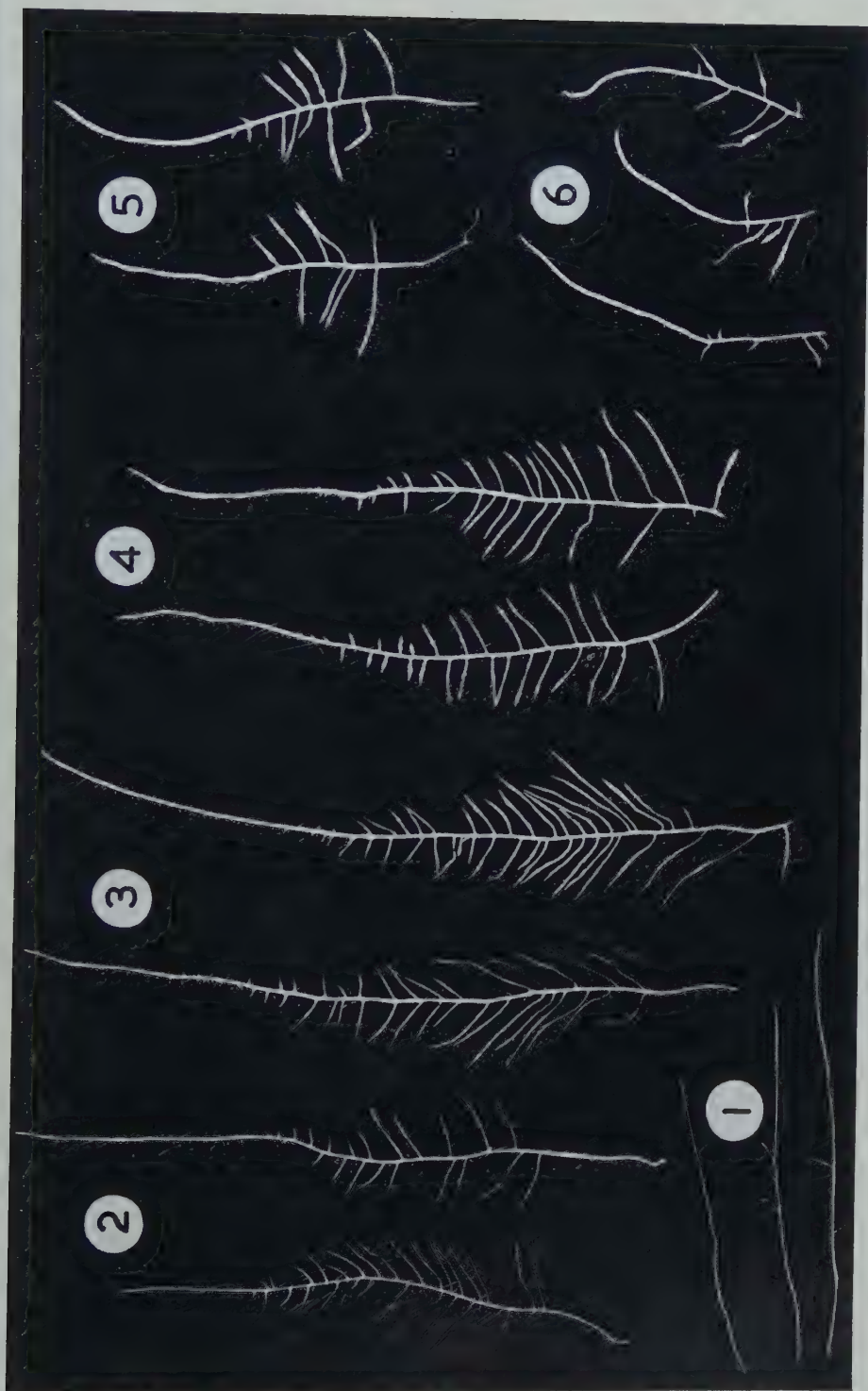
PLATE XIII

All at a magnification of $\times 150$

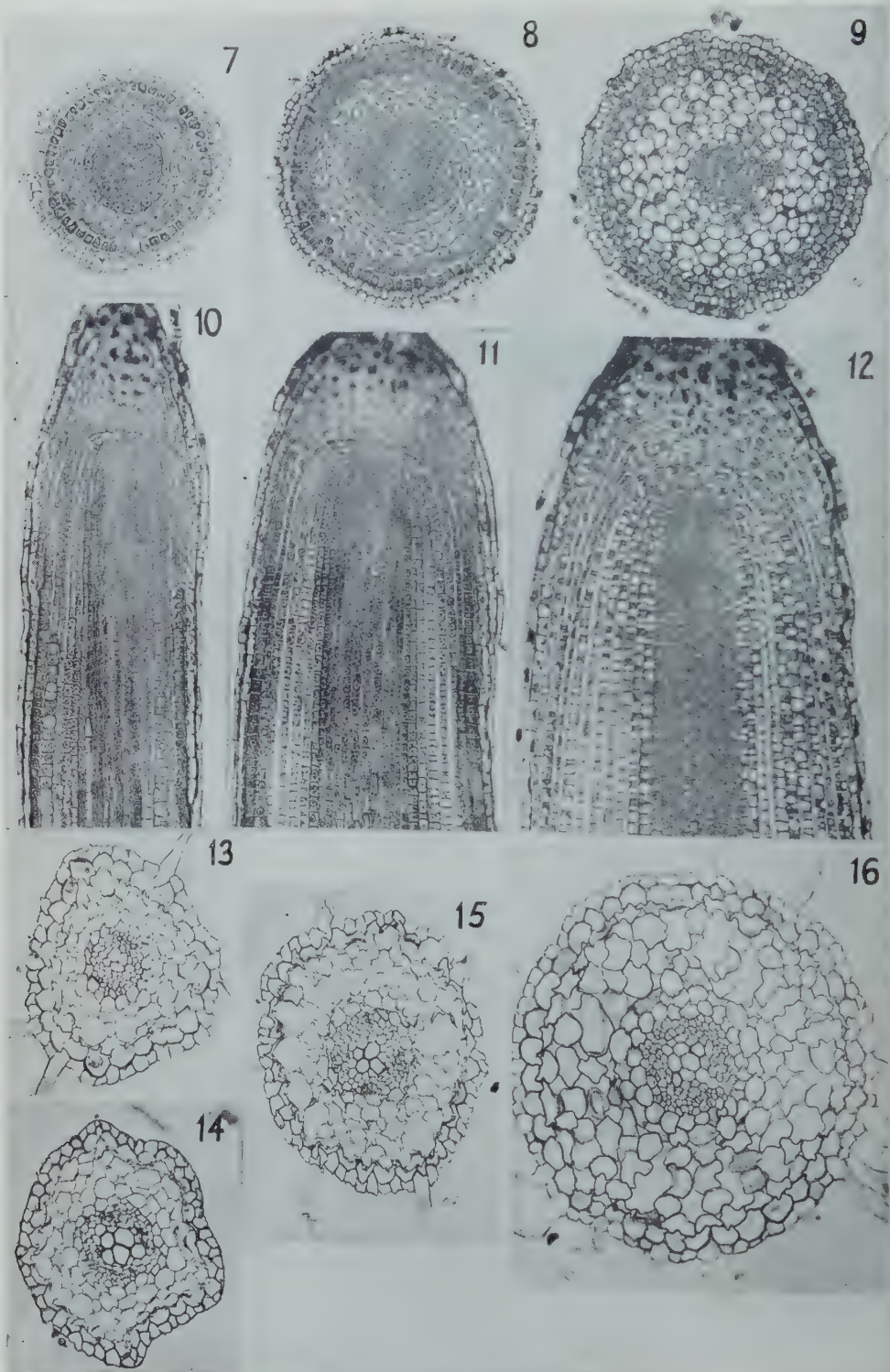
Figs. 7-9. Transverse sections 160-80 microns from the promeristem of roots grown in medium containing 0.5 per cent. (Fig. 7), 2.0 per cent. (Fig. 8), and 4.0 per cent. (Fig. 9) sucrose.

Figs. 10-12. Longitudinal sections of the root tip of roots grown in medium containing 0.5 per cent. (Fig. 10), 2.0 per cent. (Fig. 11), and 4.0 per cent. (Fig. 12) sucrose.

Figs. 13-16. Transverse sections 17-20 mm. from the root apex of roots grown in medium containing 0.5 per cent. (Fig. 13), 1.0 per cent. (Fig. 14), 2.0 per cent. (Fig. 15), and 3.0 per cent. (Fig. 16) sucrose.



H. E. STREET and S. M. MCGREGOR



Experimental and Analytical Studies of Pteridophytes

XVIII. The Nutritional Status of the Apex and Morphogenesis

BY

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With one Figure in the Text

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ABSTRACT

The relation between the nutrition of the shoot apex and its morphogenetic activity is discussed with special reference to the ferns. From an early stage in the individual development the distal region of the shoot is dependent on supplies of nutrients (the term is used in a comprehensive sense) passed up from the basal absorptive region. The obconical enlargement of the shoot is attended by an increase in the size of the apical and sub-apical regions; but whereas the primary morphogenetic activities of the apical region are closely comparable in small and large apices, very pronounced differences in leaf shape and size and in stelar morphology may be induced according to the nutritional status of the sub-apical regions. Large apices, which normally yield large and complex leaves and an elaborate vascular system, can, by appropriate treatments, be caused to yield 'juvenile' leaves and a simple vascular system. The nutritional status of the sub-apical regions is thus seen to be of considerable interest and importance in studies of morphogenesis. A conception of the mode of distribution of nutrients in the shoot apex is described and discussed. The need for physiological investigations of the apex is emphasized and the scope for new work using the methods of tissue culture is indicated by reference to recent published work.

I. INTRODUCTION

STUDIES of the embryogeny and of bud development in vascular plants indicate that, from a very early stage, the distal or apical group of cells becomes the active locus of utilization of the nutrients taken up from the gametophyte or parental tissue by the proximal, basal, or foot region. Throughout the life of the individual plant, the growth and formative activities of the apex are dependent on supplies of nutrients moving acropetally from below;

and, as the term is used here, these nutrients may include mineral nutrients, carbohydrates and other organic nutrients, and also enzymes, growth-regulating substances, &c. A knowledge of the nutritional status of the apex, in short, is essential to an understanding of its morphogenetic activity. In 1908 Goebel stated that the very marked differences in the morphology of fern leaves, from the young sporophyte, with its so-called juvenile leaves, to the large elaborate leaves of the adult, were attributable to the nutritional status of the apex, all the primordia—size differences excepted—being identical at their inception. The purpose of this paper is to consider what is meant, or implied, by the 'nutritional status of the apex' and how morphogenetic processes may be affected by it, reference being made to the shoot apices of pteridophytes and seed plants.

II. APICAL SIZE AND MORPHOLOGICAL DEVELOPMENT

A few examples will illustrate that a direct relationship probably does exist between the nutritional status of the apex and its morphogenetic activities. These relate to the elaboration of leaf form and vascular structure during the ontogenetic development.

In the ferns (Bower, 1923), the young sporophyte, or a bud rudiment, has small simple leaves. As the plant grows, the new leaves which are formed show a progressive increase in size and complexity, and the shoot undergoes a characteristic obconical enlargement, i.e. as root development and the area of photosynthetic tissue increase, the shoot apex is supplied with progressively increasing amounts of nutrients and changes take place in its formative activities. Thus the leaves of the adult plant differ markedly in shape and size from those which were produced by the young sporophyte, metabolic factors being primarily involved and the effect being essentially an acropetal one. Among seed plants the young shoot also shows obconical development and changes in leaf size and shape may occur from the base to the apex. This *heteroblastic* development has been the subject of numerous researches, recently reviewed by Ashby (1948).

The obconical development of the fern shoot is attended by remarkable changes in the vascular system. Thus, in the young sporophyte, the vascular system, which originates below the small shoot apex, is typically a solid protostele, circular in cross-section, with xylem in the centre and surrounding phloem. The small, simple leaf-traces of the first leaves become conjoined with the periphery of this stele with minimal disturbance of its configuration. But, as the sporophyte enlarges, so also does the shoot apex, and the incipient vascular tissue in the apical and sub-apical regions becomes medullated. The bases of the leaves which are now being formed also show enlargement and their vascular strands become crescentic in shape (as seen in cross-section). On the further enlargement of the shoot apex, the newly differentiated region of the vascular column is seen to be solenostelic: a more or less considerable pith may be present and internal as well as external endodermis and phloem may be differentiated; and where the deeply crescentic leaf-traces become

conjoined with the shoot stele, the latter becomes interrupted by parenchymatous leaf-gaps. If new leaves arise in close sequence and if shoot elongation is slow, i.e. if the shoot is closely beset with leaves, the shoot stele becomes differentiated as a mesh-like or dictyostelic configuration and several leaf-gaps can be seen in any cross-section. In some species the enlargement of the shoot is also accompanied by the differentiation of longitudinal panels of parenchyma in the stele, i.e. by the development of 'perforations', which are not associated with the insertion of leaves. In other species the enlargement of the shoot apex is accompanied by the differentiation of one or several concentric inner vascular cylinders. Disruption of the petiolar vascular system is characteristic of many species (Bower, 1923; Ogura, 1938). Comparable examples are also known in seed plants.

The foregoing examples show that, in the development of a fern, there is a general increase in size affecting the apex, the shoot, the leaves, and the vascular system. The converse has also been observed. Thus, by various treatments, the shoot apex can be progressively reduced in size and eventually attenuated to the point where its meristematic character disappears (Lang, 1924; Wardlaw, 1945*a*). In such plants there is a notable reduction in the size and complexity of the leaves and the stele. Thus a large dictyostele has been reduced to a small protostele and eventually the latter has faded out.

Another aspect of the relation between the nutrition of the shoot apex and its morphogenetic activity is seen in flowering plants when, under the influence of appropriate photoperiodic treatment, the vegetative shoot apex is transformed into a floral or inflorescence apex. In such cases—and here the matter is being treated in very general terms—the vegetative apex loses its dome-shaped or conical appearance and broadens out; and instead of leaves and shoot buds being formed as the characteristic lateral members, floral parts or flower buds are formed. The factors which determine these changes are not yet understood, but there is general agreement that metabolic changes, which have been induced in the more mature regions of the plant and which exercise an acropetal effect, are primarily involved.

III. GROWTH IN THE APICAL AND SUB-APICAL REGIONS

In leptosporangiate ferns, e.g. species of *Dryopteris*, *Onoclea*, or *Cyathea*, the shoot apex undergoes a very considerable enlargement during the development of the individual plant (Wardlaw, 1948). In some gymnosperms, e.g. cycads (Foster, 1943), and some flowering plants, e.g. *Nymphaea*, *Echinopsis*, *Gunnera* (Wardlaw, 1951*a*), this is also the case. In many flowering plants, however, the shoot apex probably attains to its maximum size in the young seedling and thereafter shows little change until the onset of flowering, e.g. *Lupinus* (Ball, 1949).

In the first instance, the fern apex will be considered as its clearly defined histogenic regions enable certain ideas to be set out with greater ease and clarity. Fig. 1 shows the main features of the shoot apex in *Dryopteris*. Such

an apex may for convenience be described as consisting of three regions: (i) the apical region; (ii) the sub-apical region; (iii) the maturing region (in that this region merges with the mature tissue of the shoot, it lies partly outside the apex). Some features of these *growing regions* may now be specified.

(i) *The apical region.* This is the distal or terminal, rounded or conical region of the shoot. A large inverted tetrahedral cell occupies the summit. This apical cell undergoes a regular sequence of divisions by walls parallel to its lateral sides, and thus gives rise to a superficial apical meristem, consisting of distinctive prism-shaped cells. These extend down the sides of the cone for a variable distance. The young leaf primordia originate just within the basiscopic margin of the apical meristem as thus defined (Wardlaw, 1943, 1949). The prism-shaped cells divide by anticlinal walls and also into unequal segments by periclinal walls. The smaller inner segments differ in their staining reactions from the outer ones, they undergo further divisions by periclinal walls, and the tissue thus formed is the incipient vascular tissue: farther down the shoot it undergoes further differentiation into the several tissues of the stele. Immediately below the apical cell group, the innermost cells of the incipient vascular tissue are seen to be in a state of transition, their staining reactions being those of young developing parenchyma. These transitional cells (which are approximately the equivalent of the rib-meristem in dicotyledon apices) continue to divide and give rise to the parenchymatous cells of the pith. The apical region is thus seen to be *the* primary formative region: it maintains itself during the life of the plant, it gives rise to the lateral organs and to the incipient tissue systems. Since all the cells of this region are in an actively meristematic condition, it must be a locus of active protein synthesis and of some carbohydrate utilization. During quiescent periods starch may be deposited in the apical region as well as in the more parenchymatous regions below.

(ii) *The sub-apical region.* The characteristic and evident feature of this region in ferns is the considerable increase in the diameter of the shoot, the basal region of the apical cone becoming distended laterally, i.e. radially. These developments are due to the great enlargement of the pith, to the inception and enlargement of the cortical parenchyma, and to the distension of the bases of the enlarging leaf primordia. In the upper limits of this region the vascular tissue is still at the incipient stage, but towards the lower limits the incipient phloem and xylem elements can be distinguished.

The enlargement of the pith is due to the further division of the pith mother cells and their products. The cortical parenchyma has its inception on the abaxial side of the young leaf primordia. It arises by numerous periclinal and anticlinal divisions of the prism-shaped meristematic cells along the basiscopic margin of the apical meristem. This formation of parenchyma in the cortex, as also in the leaf-bases which merge with the cortex, takes place quickly: hence, in conjunction with the enlargement of the pith, there is a notable increase in the diameter of the shoot in the sub-apical region.

There is also an increase in the length of this region. This may be slight or considerable in different instances.

As the growth of the apical region continues, the leaf primordia to which it has given rise come to occupy positions in the sub-apical region, where they undergo rapid growth and development and acquire their characteristic dorsiventral symmetry. Moreover, it is in this region that their *major* morphological features become evident, both general nutritional and more specific genetical factors being at work.

The sub-apical region, with its abundant development of parenchyma and, in quiescent periods, of starch deposition, is sometimes regarded as one in which carbohydrate metabolism is predominant. This view needs to be qualified, for, as we have seen, the region is one in which there is a great deal of meristematic activity. Hence, in the formation of protoplasm in the enlarging epidermis, cortex, stele, and pith, there must be extensive protein metabolism—an aspect that perhaps tends to be obscured by the extensive vacuolization of cells. Vacuolization, the formation of thick cellulose walls, and deposition of starch are, of course, all indications that carbohydrate metabolism is a feature of the sub-apical region in a way that it is not a feature of the apical region. In some ferns, e.g. *Cyathea* and *Pteridium*, the parenchymatous development in the sub-apical region is so extensive that that region actually bulges upwards, the apical region being left in a more or less conspicuously sunken position.

(iii) *The maturing region.* This region cannot be precisely defined, but it may be indicated as the region where the developing but still immature tissues of the sub-apical region enter on the final phase of their enlargement and maturation. In a longitudinal section it is seen to be that region where the shoulders or flanks of the sub-apical region are becoming finally distended and the full width of the mature, rigid part of the shoot attained. In this region the cells of the epidermis, cortex, and pith are reaching their maximum size and those of the stele are becoming fully differentiated, lignified, &c. Lower down are the mature tissue systems of the rigid shoot and leaf-bases. At this level the leaves are still growing actively by their apical and marginal meristems. Because of the more rapid growth on their abaxial side, these leaves, which now over-top the shoot apex, show the characteristic circinate vernation. It is at, or soon after this stage that leaves begin to show specialization in various ways, e.g. to become transformed into cataphylls, as in *Osmunda*, or to become sporogenous members.

In the apices of flowering plants the same *general* dispositions can be discerned, but there are considerable differences in detail both between the apices of flowering plants and ferns and among flowering plants themselves. Two main differences between the apices of flowering plants and ferns are that flowering plants do not show (i) such a clearly defined apical meristem, and (ii) the procambium (or incipient vascular tissue) in the close relationship to the apical meristem that is readily demonstrable in ferns. With these reservations, however, the apices in the two classes have much in common

(Wardlaw, 1951). In a typical zoned apex, such as that of *Primula*, or *Phaseolus*, the tunica cells occupying the summit of the shoot and comprising the last-formed leaf primordium may be accepted (on a purely arbitrary basis for purposes of description and discussion) as constituting the apical meristem. Immediately below the tunica, at the summit of the shoot, there are actively dividing cells, usually described as the rib-meristem, which give rise to the pith. In favourable materials such as *Trichocereus*, as described by Boke (1941) or *Echinopsis* (Wardlaw, 1951a) procambial tissue can be seen at the junction of the tunica and the corpus and extending upwards towards the apical meristem. Since it is almost certain that there are related metabolic processes antecedent to the visible appearance of the procambium, then, by extrapolation, this tissue would have its inception just below the apical meristem as in the ferns. An organization of this kind would appear to be present in those dicotyledon apices in which a 'provacular ring' or a 'residual meristem' has been observed. On some such basis as that now indicated, an apical region can be distinguished in flowering plants which is physiologically the equivalent of what we find in the ferns; while the sub-apical and maturing regions also show developmental features which are comparable with these regions in the ferns.

IV. MORPHOGENESIS IN THE APICAL AND SUB-APICAL REGIONS

As already noted, the *newly formed* leaf primordia in the young fern sporophyte and in the adult plant are closely comparable, and it is a reasonable implication that comparable morphogenetic activities are involved. But the *fully formed* leaves, in the two instances, differ greatly in size and shape. These differences have their inception in differences in the growth development of the primordia in the sub-apical and maturing regions. In the young sporophyte the sub-apical region is of small diameter and comprises a central protostele surrounded by a narrow cortex, whereas the equivalent region in a stout shoot has a wide cortex surrounding a large medullated stele. Again, ferns such as *Dryopteris aristata* and *Matteuccia struthiopteris* have both stout, erect shoots, and narrow horizontal underground rhizomes or runners. The histological details of leaf inception are identical in both shoot and rhizome. But whereas the erect shoot, with its wide sub-apical region, gives rise to large foliage leaves, with stout petioles, the rhizome, which is entirely dependent on the parent shoot for its organic nutrients, typically gives rise to feebly or non-laminate, fleshy scale-leaves. In due course such a horizontal rhizome may appear above-ground and develop as an erect shoot. Some remarkable changes then ensue: progressively larger foliage leaves are formed, so that a local source of organic nutrients becomes available; there is a rapid increase in the diameter of the shoot and in the size of the whole apical region. In short, the diameter of the shoot and the size and shape of the leaves are largely determined by the nutritional status of the sub-apical region, the primary morphogenetic developments which take place in the apical meristem remaining virtually unchanged. (The fern leaf, of course,

continues to grow by its apical and marginal meristems for some considerable time.) The facts of stelar development bear out these relationships: for example, it is mainly in the sub-apical region that the characteristic tissue pattern of a dictyostelic shoot is established (Wardlaw, 1945). Furthermore, if large shoots are subjected to defoliation or to 'starvation' conditions, there is a reduction in the size of the sub-apical region, in leaf size, and in stelar complexity.

There is yet another respect in which the sub-apical region is important in morphogenesis; for it is in this region that some of the distinctive features which characterize genera and species begin to be manifested, i.e. the effects of certain genetical factors become evident in this phase of development, just as others become evident in the final maturation of organs and tissues. It is in the sub-apical region, for example, that the main differences between the leaves of *Dryopteris* and *Angiopteris* begin to be apparent; and similarly, in dicotyledons, it is in this region that one can begin to distinguish the features that characterize the leaves of *Primula*, for example, as compared with those of *Phaseolus*, *Heracleum*, or *Iris*.

V. SUPPLY OF NUTRIENTS TO THE APEX

From an early stage in the embryogeny and throughout the growth of the shoot, the apex is supplied with all its nutrients from below, i.e. from the root system, the older functional leaves, and storage reserves in mature regions of the shoot. It is assumed that, in the fully differentiated regions of the shoot, the translocation of nutrients to the apex is by way of the phloem and xylem. These specialized tissues fade out, however, some distance below the apical region, i.e. in what has been designated here as the region of maturation, the sub-apical and apical regions being traversed by the small-celled incipient vascular tissue only. In view of the relation that has been established between the nutritional status of the apex and morphogenesis, it is cogent to inquire into the distribution of nutrients.

Fig. 1 illustrates a conception of the distribution of nutrients at the shoot apex of *Dryopteris*. With only minor leakages, the nutrients moving upwards are retained within the mature vascular tissues. Towards the upper limits of the region of maturation, *region iii*, the endodermis and vascular elements are incompletely differentiated and an outward, inward, and upward diffusion of nutrient solutions from the vascular column takes place, the nutrients moving along concentration gradients. These gradients, however, do not operate in a uniform tissue mass, for, as we have seen, the cells become progressively more dense and meristematic towards the apex. In the growth and maturation of any cell there are two variables which must be considered here: (a) initially the cell will have the properties of the meristematic cell from which it was derived, but these properties will gradually change during differentiation and maturation; (b) initially the cell, situated close to the apex, will be at a low point on the concentration gradient but it will subsequently occupy positions which are progressively farther away from the apex and

therefore higher on the concentration gradient. Thus, a young pith cell in *position A*, Fig. 1, a product of a recently divided meristematic cell, occupies a position remote from the source of nutrients; but, as a result of the continued growth of the apex, it may eventually occupy *position B*, i.e. close to the source of nutrients. At this level it will be an almost fully grown, vacuolated cell. On further growth it may occupy *position C* and will there be recognized as a fully developed, vacuolated parenchymatous cell, constituting part of the rigid structure of the mature shoot.

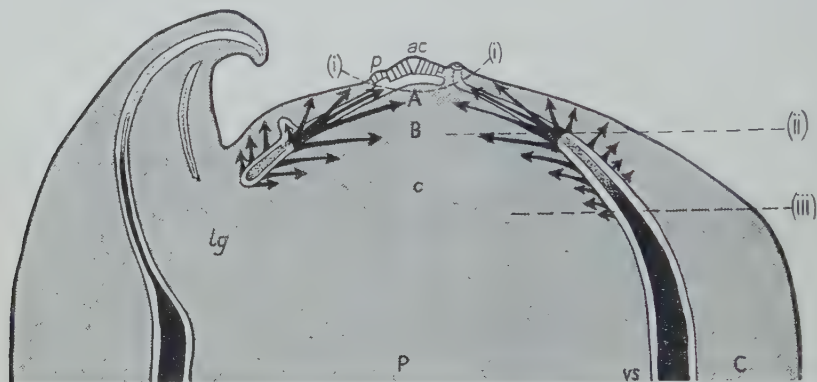


FIG. 1. Diagrammatic longitudinal median section of a large shoot apex of *Dryopteris aristata*. The following growing regions are indicated: (i) the apical region; (ii) the sub-apical region; (iii) the maturing region. The arrows indicate a conception of the distribution of nutrients in the three regions. *ac*, the apical cell, flanked by prism-shaped cells of the apical meristem, which includes the last formed leaf primordium, *p*; *P*, pith; *C*, cortex; *lg* leaf-gap; *vs*, vascular strands. ($\times 10$).

As already noted in Section III, the apical region is in the main characterized by protein metabolism and tends to continue in the meristematic state, whereas in the sub-apical regions carbohydrate metabolism tends to supervene upon the initial protein metabolism, with concomitant vacuolization and parenchymatization of the embryonic cells. Now, when embryonic cells are supplied with metabolites, including auxin and abundant supplies of sugar, they typically undergo rapid enlargement and vacuolization, and this continues as long as the cell is free to expand. This may account for the typical parenchymatous development of cells in greatest proximity to the source of nutrients, e.g. at positions B and C in Fig. 1. In these enlarged and maturing cells, which may continue to utilize carbohydrates for some time, e.g. in the formation of thick cellulose walls and deposition of starch, there will be a diminution in the utilization of nitrogen-containing metabolites. Hence those embryonic cells which are distant from the source will receive proportionately more nitrogen-containing metabolites and less sugar—an arrangement which will tend to maintain active protein synthesis and perpetuate the meristematic character of the most distal, i.e. the apical region. Here it is relevant to note that when apical growth ceases with

the onset of winter conditions, a progressive acropetal deposition of starch takes place.

The foregoing conception of a differential utilization of carbon and nitrogen metabolites in the sub-apical region, with a consequential relatively low C/N ratio in the distal meristem is, of course, no more than a broad and tentative and, no doubt, over-simplified sketch of the distribution of nutrients in the growing region. Other factors may also be at work in maintaining the characteristic protein metabolism of the most distal region of the shoot. For example, some nutrients, e.g. nitrates, may diffuse or otherwise move in the tissues of the sub-apical region much more rapidly than carbohydrates. If so, this might partly account for the extensive nitrogen metabolism in the apical region. Other factors may also be involved. Thus van Overbeek *et al.* (1941, 1942) have shown that a factor in coconut milk makes for a vast proliferation of the densely protoplasmic meristematic cells in young embryos of *Datura*.

In an enlarging rhizome or shoot, the pith, which may be of small cross-sectional area or completely absent at the thin base of the shoot, shows a progressive increase in size. Thus, the protostelic stolon of *Nephrolepis cordifolia* widens out into a tuber with a dictyostelic vascular system. There is a fortyfold increase in the cross-sectional area of the shoot, and the ratio of the cross-sectional areas of cortex/pith is 1.26; in still larger tubers the areas of pith and cortex may be equal (Wardlaw, 1945). Studies of the development of pith and cortex from base to apex in fern shoots, e.g. those of *Dryopteris* and *Cyathea*, show a progressive decrease in the ratio $\text{area Cortex}/\text{area Pith}$. In stout shoots of *Cyathea imrayana*, $aC/aP = 1.7$; in *Cyathea medullaris* (in which accessory medullary strands occur) $aC/aP = 0.64$; in the large solenostele of *Cibotium Barometz*, $aC/aP = 1$; and in *Dryopteris aristata*, $aC/aP = 1.6$ in the thickest region of a stout adult shoot (Wardlaw, 1945). In short, except in very large shoots aC is greater than aP . Moreover, as the appropriate measurements at the apex show, the cortex attains to its maximum size earlier than the pith. In other words the growth of the cortex slows down while that of the pith is still active. Among contributory causes, the fact that the outer surface of the vascular cylinder is of greater area than the inner surface may be important; these are in the ratios of 1.8 and 1.2 in the apical and maturing regions respectively. These examples show how necessary it is to have concepts relating to the distribution of nutrients at the apex in the study of morphogenesis. The views now advanced are not incompatible with the facts of observation.

Recent studies by Wetmore *et al.* (1949, 1950, 1951) have shown that there is a basipetal movement of auxin from leaf primordia and petioles into the shoot. Together with the metabolites diffusing out from the vascular system, this auxin may be a factor in the development of parenchyma in the cortex, leaf-gaps, and pith (see below). Because of the superficial position of leaf primordia, there may be a differential growth effect as between the cortex and the pith, the former showing the greater initial response. The wide parenchymatous

leaf-gaps in ferns are closely associated with, and determined by, the considerable tangential growth of the pith in the leaf-bases (Wardlaw, 1944). Here again auxin, produced by the leaf primordia, is probably a factor in the situation; for the incipient vascular cylinder of the shoot is disrupted at the point of insertion of a leaf-trace, and the affected cells not only undergo parenchymatization, but they continue to grow and divide so that a large parenchymatous gap is formed.

In leptosporangiate ferns such as *Dryopteris*, young pith cells can be observed just below the apical cell and only separated from it by a thin sheet of incipient vascular tissue. The presence of a pith is not necessarily associated with foliar development, e.g. a pith is present in the swollen, leafless stolons of *Nephrolepis*, in the leafless shoots of *Psilotum*, and in the microphyllous shoots of *Equisetum*. In short, although in many plants the formation of pith parenchyma may be due in part to auxin from foliar sources, there are others in which the auxin must come from the apical meristem. If we assume that a fern apical cell group, whether of shoot or leaf, produces some free auxin we have a hypothesis which helps to explain why some medullary parenchyma is formed just below the apical cell and above the youngest leaf primordia, and also why the whole shoot undergoes such a remarkable increase in girth, largely due to the formation of parenchyma, below the top cycles of leaf primordia.

Assays of the auxin diffusing out of the fern leaf-base have been made by Wetmore and Pratt (1949). They have shown that (i) there is active auxin production in the unrolling leaves, (ii) the amount decreases in successively younger leaves, and (iii) no auxin is detectable in the shoot apex and youngest leaf primordia by existing methods. From this it may perhaps be inferred that, in meristematic regions, auxin is an effective morphogenetic agent at very low concentrations, or that some other highly active growth-regulating substance, not yet detected, is produced by the apical meristem. The occurrence of long and short shoots, as in *Ginkgo biloba* (Gunckel and Thimann, 1949) and other plants, is also referable to the nutritional status of the apex: in these instances the supply of auxin, or some other growth-regulating substance, is of critical importance.

VI. EXPERIMENTAL INVESTIGATIONS OF ORGANOGENESIS

As we have seen, the inception of leaves in the ferns takes place in a remarkably constant manner throughout the development of the individual; but their further development may be more or less notably affected by the nutritional status of the sub-apical regions. In the normal development of the sporophyte, the leaves show a progressive increase in size and complexity, but a return to the 'juvenile' leaf condition has been effected in 'starvation' and other experiments (Wardlaw, 1945a). An extreme example is seen in the awl-shaped leaves as described by Lang (1924) in *Osmunda*, some of the very 'reduced' leaves giving rise to prothalli. In experiments with large *Dryopteris* apices, young leaf primordia were isolated by four vertical incisions. These

primordia showed very limited growth and became typical awl-shaped parenchymatous structures (Wardlaw, 1949). These and other examples testify to the important relationships that exist between nutrition and morphological development. Such observations, however, are still of a very general kind. What is now required are detailed physiological studies, under controlled environmental conditions, in which the general concept of 'nutritional status' can be particularized in terms of the principal metabolites involved. In this connexion the methods of tissue culture promise to be of great value; for environmental factors can be controlled and the effects of particular nutrients on leaf and shoot development can be ascertained with some exactitude by varying the composition of the medium. Thus Allsopp (1951, 1951a) has shown that remarkable differences in rhizome and leaf development can be induced by varying the quantity of sugar and other metabolites supplied to young plants of *Marsilea*: in one medium, normal laminate leaves are formed: in another only non-laminate, awl-shaped leaves. Comparable data have accrued from tissue culture investigations by Wetmore (1950). Thus far, in studies of organogenesis by the methods of tissue culture, one outstanding result has been obtained, namely, that while the rate of growth and development, and the relative sizes of different organs can be modified by providing different cultural conditions, the fundamental morphogenetic activities by which the species is characterized remain singularly unchanged, i.e. the sub-apical regions are those which are chiefly affected.

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Investigations on the Growth and Metabolism of Plant Cells

II. Variables affecting the Growth of Tissue Explants and the Development of a Quantitative Method using Carrot Root

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AND

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With Plates XIV and XV and nine Figures in the Text

ABSTRACT

This paper describes the technique of growing small discs of carrot phloem in pure culture and with the precautions necessary to standardize the rate of growth at the highest practicable level. It deals with the procedure necessary to remove replicate samples of the material, with variability along the radial and longitudinal axes of the carrot, and with the regulation of the culture conditions.

Evidence is adduced of the existence of a 'staling factor' in crowded cultures.

THE culture of excised plant tissues is playing an increasing role in the investigation of normal and abnormal growth (White, 1943; Gautheret, 1947; Nobécourt, 1948). After Gautheret (1939) and Nobécourt (1939) described the growth of explants of carrot root under the influence of indole-3-acetic acid, and White described the more autotrophic growth of excised tissue from a tobacco hybrid capable of producing tumours spontaneously (White, 1939), the potentialities of the plant tissue culture technique as a general research method were evident.

But progress was relatively slow. It is true that new tissue cultures from other plants were obtained (Gautheret, 1947; Nobécourt, 1948), some from bacterially induced tumours sterilized by heat (Braun, 1947); also tumorization became recognized as a distinctive phenomenon (Braun, 1947). However, certain limitations in the tissue culture technique precluded its wide adoption as a research method generally applicable in cell physiology. Among these limitations was the relatively slow growth achieved, which commonly necessitated many weeks or months (Gautheret, 1939; Hildebrandt et al., 1945; White, 1939) before the results of a given treatment became known. Furthermore, the variability inherent in the continuously subcultured strains of tissue cultures, which have been the staple materials employed in this field,

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presented difficulties in quantitative experiments. It is true that some tissue cultures, e.g. marigold (Hildebrandt et al., 1949) were able to grow relatively rapidly, but their growth was unpredictable since many subcultures were unable to grow at all.

The technique of subculturing was a potent source of variation since they were neither uniform in size nor identical in morphology. In one laboratory subcultures varied from 20 to 30 mg. when cut with a scalpel (Hildebrandt et al., 1945). Affecting the potential uniformity of subcultures from many tissues (e.g. *Vinca*) is the presence of irregular knots of vascular tissue, while others are so friable (e.g. *Helianthus*) as to defy accurate subdivision. Even tobacco cultures uniformly cut into 8 mg. grew at very different rates (Caplin, 1947). At the end of 8 weeks, for example, the fresh weight of subcultures from the same source varied over a tenfold range. Such wide differences are clearly the expression of many factors, some of which are inherent in the morphology of the original cultures and others of which are inherent in the growth of the subcultures as determined by their environment.

The plant tissue culture technique has therefore been re-examined with the following objectives: (i) To find a readily obtainable material from which uniform explants could be removed aseptically. (ii) To devise procedures such that each of the explants would grow under standard conditions at the same and preferably rapid rates within narrow limits. (iii) To discover a tissue system in which the cells could be placed reversibly and at will in the condition in which they would either grow by rapid proliferation or merely remain alive in the relatively inactive state.

Certain potential sources of tissue cultures were tested with each of a number of substances reported to stimulate proliferative growth. The plant materials tested included: (i) Standard pieces of dormant potato tuber, the cells of which are capable of growth as shown by phellogen formation. (ii) Explants from carrot root (both xylem and phloem). (iii) Tissue from the floral receptacle of crab apple (*Pyrus* sp.) and from the ovary wall of cherry (*Prunus cerasus*), the cells of which are commonly not able to divide again once they are mature, as well as entire pistils from unopened flowers of May apple (*Podophyllum peltatum*); all these organs being normally stimulated to develop by the presence of an embryo. (iv) The leaf base of Narcissus (*N. tazetta*), a monocotyledonous tissue normally able to grow in the intact plant and capable of limited extension even when isolated. (v) The inner epidermis of young bean pod (*Phaseolus vulgaris*, 'Kentucky Wonder', brown-seeded variety), a translucent tissue which develops by an irregular meristem, and is reputed to be stimulated into rapid growth by certain plant extracts and by the substance traumatin of English and Bonner (1937 and 1939).

Explants from these materials were each inoculated on White's (1943) agar media containing one of the following substances, each at several concentrations: (i) Traumatin (1-decene 1,10 dicarboxylic acid), reported to initiate both cell division and cell elongation in the inner epidermis of the bean pod (English et al., 1939). (ii) Phenobarbital, used by Curtis (1947) on the orchid

embryo to transform it into an undifferentiated proliferating tissue mass. (iii) Stilbesterol, a synthetic estrogen, utilized by Swarbrick (1945) to induce parthenocarpic development of tomato and apple, and thus to stimulate the effects of the embryo on somatic tissue. (iv) 2,4-D (2,4 dichlorophenoxyacetic acid), well known (Swanson, 1946; Tukey et al., 1945) to have a profound effect in stimulating growth, particularly of the cambium. (v) Coconut-milk, the liquid endosperm of *Cocos nucifera*, the nutritive fluid which nourishes the immature embryo of the coconut on germination, used by Van Overbeek et al. (1941) to culture immature *Datura* embryos, and by Ball (1946) to culture portions of excised stem tips of *Tropaeolum*. (vi) I.A.A. (indole-3-acetic acid), the natural auxin, widely used in growth and metabolic studies as well as in the culture of excised carrot root and other plant tissue (Gautheret, 1939; Nobécourt, 1939). The effects of these various combinations of plant material and growth substance will not be reported in full: there were, however, two which yielded particularly interesting results.

First, there was a limited response of potato tuber tissue to 2,4-D. A 9-mg. cylinder of cortical tissue grew to 45 mg. in a nutrient medium containing an optimum concentration (7 mg. per litre) of 2,4-D, but growth then stopped. Subsequently (Steward and Caplin, 1951) a continuously growing culture of potato tissue was obtained, but the investigation of this was for the time deferred because of the second outstanding effect, which was obtained with carrot tissue. After 46 days the mean fresh weight of carrot-root explants increased from 9 to 487 mg., or 54.1 times. It has been shown that the substance in the coconut-milk responsible for this response is not indoleacetic acid (Caplin and Steward, 1948).

Therefore, the system composed of carrot-root explants in media with and without coconut-milk had many of the desired properties of a tissue culture for a variety of physiological experiments.

Excised plant tissues are usually grown on agar. However, a technique has been devised by which they can be grown in liquid media. This eliminates some of the sources of variation inherent in growth on agar (Steward, Caplin, and Millar, 1951).

This paper describes in detail the cultural and other methods which give the maximum and most uniform growth of carrot-root explants using the new procedure referred to, as well as certain salient experiments which indicate the nature of the additional factors which need to be controlled.

REMOVAL OF UNIFORM CYLINDERS ASEPTICALLY FROM CARROT ROOT AND THEIR TREATMENT

The basis of the technique is the aseptic removal of explants from mature carrot roots. Except for special purposes these are not continuously subcultured, since cultures are established anew from a different carrot for each experiment. The reasons for certain of the steps by which uniform cylinders with maximum growth capacity are obtained will be apparent from the experiments to be described later.

After washing a large carrot root, the upper inch or so, i.e. hypocotyl (Esau, 1940), is removed. If the cambium appears circular in outline and is clearly defined (Pl. XIV, Fig. 1) the carrot is peeled and wrapped in paper towelling, leaving one end open. Sufficient 1:16 'Chlorox' (commercial NaOCl diluted with water) to wet the paper completely is poured into the open end of the wrapping. The carrot may then be carried aseptically into the transfer room, which has been previously treated with triethylene glycol¹ (Robertson, 1947; Peck, 1947) or other suitable substance. In the room the carrot is unwrapped and placed aseptically in a dish. Using a sterilized (alcohol dipped and flamed) surgical cannula, as many radial cylinders are removed from the carrot as are needed, and these are placed in a covered sterile Petri dish. (Cannulas of 2.0 and 1.8 mm. diameter have been used, the former yields explants weighing 3.0 mg.) The cylinders are best obtained from an inch or two along the upper part of the carrot root. If the cambium is not perpendicular to the axis of the cut cylinders they are discarded.

Ten such cylinders are moved with the point of a knife (Pl. XIV, Fig. 1) to lie similarly side by side in the Petri dish and are arranged so that the cambium is exactly aligned (Fig. 1). (Half-cylinders broken or cut in the xylem, but with the cambium and phloem intact, may, of course, also be used.) In this position the phloem is ready to be cut into standard cylinders.

The cutter consists of four 'Personna' or other suitable thin, stainless steel razor blades separated by aluminium spacers: the combined thickness of one blade and spacer is 1 mm. The spacers cover each blade to within 3 mm. of its edges. Blades and spacers are rigidly held by bolts (Pl. XIV, *a, b*), the length of which may accommodate 3 (the number usually used for carrot) or any larger number of blades up to about 10. In use the cutter is held over the phloem with the blades perpendicular to the carrot cylinders and the edge of the first blade placed precisely on the cambium region.

Pressure will then cut off 3 rows of 10 cylinders, each at a definite distance from the cambium. After a partial cut has been made it is desirable to examine the cylinders embedded in the blades, discarding any which were improperly aligned, before completing the cut. In this work only cylinders with their inner tangential surface 1 mm. from the cambium were used. This (the middle) row of cylinders may be removed with the tip of a pointed knife into a sterile Petri dish. The cylinders so cut have circular tangential faces 2 mm. in diameter and they are 1 mm. long in the radial direction of the carrot root.

As a further precaution to maintain sterility, the cut cylinders are rinsed with a few millilitres of sterile distilled water in a covered Petri dish. The cylinders are now ready for aseptic transfer to sterile culture vessels using long, narrow, alcohol-flamed forceps.

In this way an experienced operator can remove some 100 to 150 standard explants from a single large carrot with negligible contaminations.

¹ The transfer room is first made saturated with water vapour. The triethylene glycol is evaporated from a hot plate and allowed to settle.

The essential part of this technique is that it secures for experimental purposes tissue cultures of exactly known origin. At 1-2 mm. from the cambial surface such radial cylinders contain only secondary phloem and they are composed of cells far enough from the cambium to have differentiated, and they would not normally divide again.

The growth curve of carrot explants in presence of coconut-milk and under the conditions designed for this work has been described (Caplin and Steward, 1948; Steward, Caplin, and Millar, 1951). Until the cultures reach the inflexion point on the sigmoid curve, i.e. when they may have increased from 3 mg. to 60-100 mg. or more, their growth may be used to compare different treatments within one group of experiments using explants from the same carrot.

THE CULTURE MEDIA

The basal medium used is White's (1943) nutrient solution containing salts, sucrose, and vitamins, as originally employed for tomato-root cultures and for tissue cultures, but substituting for ferric chloride an equivalent amount of ferric tartrate (5 mg. per litre). A complete basal medium is prepared at double strength from stocks of the individual nutrients. When diluting this solution (1:1) the basal medium may be supplemented by the coconut-milk. The pH of the medium is adjusted to 6.4, since experience has shown that the pH of the solution tends to drift towards this value as the cultures grow.

The coconut-milk growth factor. The growth factor (C.M.F.) for carrot tissue, which is in coconut-milk, has also been found in other sources, e.g. immature grains of corn (*Zea mays*; Caplin and Steward, 1948). The distribution of this factor has been briefly discussed (Steward and Caplin, 1950). Reference will be made here, however, only to the necessary considerations to prescribe and justify the use of coconut-milk in the growing of plant tissue cultures.

Heat stability of the growth factor. It is fortunate that the particular growth factor in question in coconut-milk is thermostable, so that the necessity for Seitz filtration does not arise and the media can be heat-sterilized. Fig. 3 shows that even after 8 hours of continuous autoclaving at 15 lb. per sq. in., 57 per cent. of the original activity remained as compared with coconut-milk which had been autoclaved for only 15 minutes, which is the routine practice.

Concentration of coconut-milk in the culture medium. Fig. 4 is a composite of five experiments. For comparative purposes the maximum growth obtained in each experiment is designated as 100 per cent., and the relative growth at each coconut-milk concentration is expressed as a percentage of the maximum. As the data show, the optimum concentration of bulked samples of coconut-milk for the growth of carrot explants lies between 10 and 20 per cent. by volume of the culture medium.

Each new batch of coconut-milk always requires to be assayed to determine its activity. Usually, however, the optimum concentration in the medium is

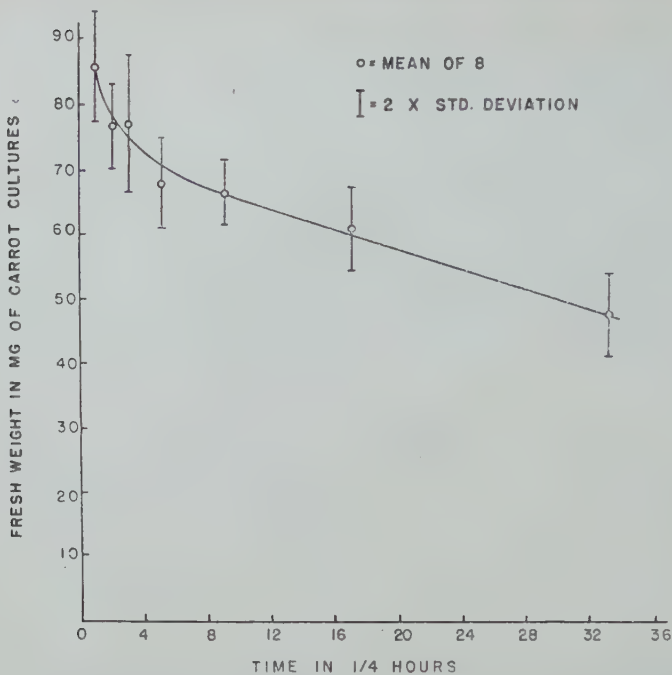


FIG. 3. Effect of continuous autoclaving on the stability of the coconut-milk factor.

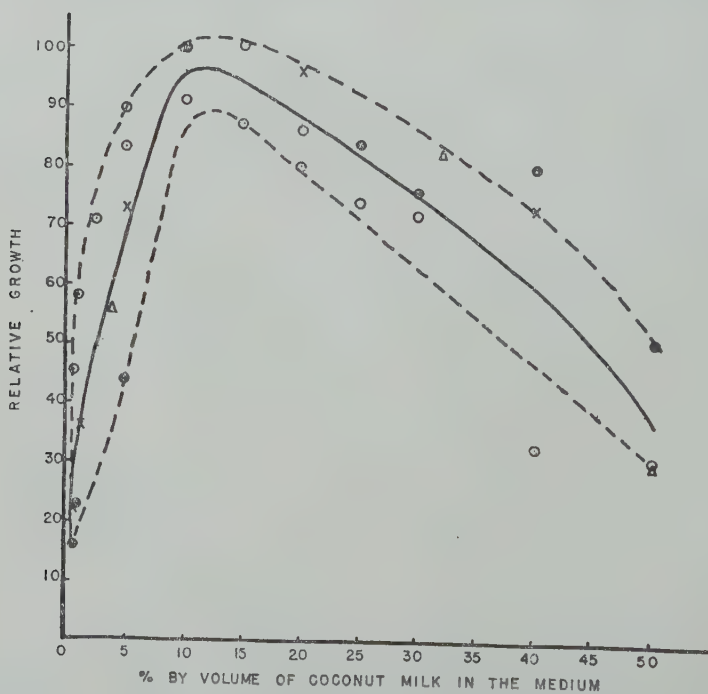


FIG. 4. The effect of concentration of whole coconut-milk on the growth of explants (3 mg.) of carrot-root phloem under standard conditions.

15 per cent., but it is sometimes more, sometimes less: in one batch the optimum was as low as 5 per cent.

It will be noted that an excess of coconut-milk depresses growth: this is not due to sugar, &c., for it also occurs with purified preparations of the growth factor.

Growth on coconut-milk alone. The growth of carrot-root explants is reduced by only a small amount when the basal medium (except for sucrose) is omitted and the cultures are grown only in 15 per cent. coconut-milk. See Table I. (It is known, however, that the general nutrients of coconut-milk are not responsible for its great activity because the isolation of the active principle is already far enough advanced¹ so that one may be sure that it is not one of the nutrients present in bulk.)

TABLE I

Growth of Explants from Carrot Root on 15 per cent. Coconut-Milk with and without Basal Nutrient Medium

						Plus basal medium.	Minus basal medium.	
Mean	.	.	.	.	.	98.0 mg.	84.7 mg.	} Expt. A*
δ	.	.	.	.	.	8.6 mg.	1.5 mg.	
Mean	.	.	.	.	.	75.8 mg.	57.3 mg.	} Expt. B*
δ	.	.	.	.	.	10.5 mg.	12.9 mg.	
δ—Standard deviation.								

* Expts. A and B used explants from different carrots.

Storage of coconut-milk in bulk. The storage of sterilized coconut-milk has an important bearing on the results obtained. When the potency of autoclaved coconut-milk became suspiciously low during long storage, experiments were made to test what manner of storage was desirable. Samples of autoclaved coconut-milk were stored for 6 months during two successive years, on a shelf at room temperature and at -20°C . Table II shows that prolonged storage of coconut-milk at room temperature reduces its activity, but in storage at low temperatures the activity is retained wholly or in large part.

TABLE II

Effect of Method of Storage of Coconut-Milk on the Growth-promoting Activity for 3-mg. Explants from Carrot Root

Storage temperature						-20° C.	About 24° C.	
Mean	.	.	.	.	.	71.7 mg.	19.3 mg.	} Expt. A*
δ	.	.	.	.	.	4.0 mg.	1.5 mg.	
Mean	.	.	.	.	.	68.8 mg.	43.6 mg.	} Expt. B*
δ	.	.	.	.	.	11.9 mg.	16.0 mg.	
δ—Standard deviation.								

* Expts. A and B used explants from different carrots.

¹ Work of E. M. Shantz in association with one of us (F. C. S.) under grant from the National Cancer Institute, Institutes of Health of the U.S. Public Health Service.

THE EFFECT OF ORIGIN OF THE EXPLANTS IN THE CARROT ROOT ON THEIR GROWTH

A number of questions arise: To what extent is the subsequent behaviour of the explant a function of its original position in the carrot? What controls the variability in the growth of uniform explants from a single carrot? How do explants from different carrots of a particular variety compare with respect

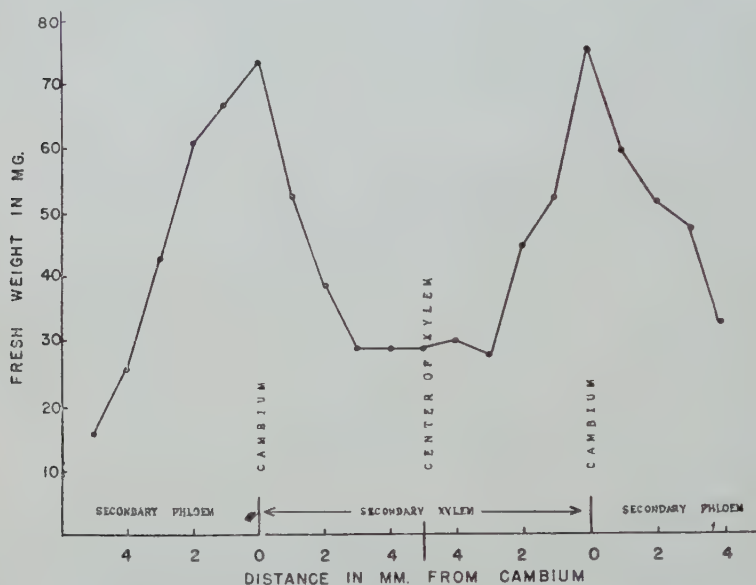


FIG. 6. The effect of distance from the cambium on the growth in 14 days of carrot-root explants (3 mg.) in media containing coconut-milk.

to their potentiality for growth? How do carrots from different varieties compare?

The increase of fresh weight of explants is a convenient criterion of their growth. However, certain small isolated carrot explants may show a limited increase in size by mere swelling and enlargement of cells already formed, in ways which do not entail external nutrition. This is evident from the following observations:

If radial cylinders through a carrot root are removed and placed in water the young phloem swells in a characteristic manner, whereas the xylem swells relatively little. Tissue rather far from the cambium (of the order of 3 mm.) does not swell appreciably. In the region of the cambium, however, the tissue swells asymmetrically. Viewed in the transverse plane of the root (Pl. XV *a*) evident swelling occurs in the region of the cambium in the tangential direction: viewed in the radial-longitudinal plane (Pl. XV *b*) no such swelling can be seen. In other words, the cells of the cambial and adjacent regions have enlarged but not elongated. The consequence is that cylindrical

explants, cut so that their plane surfaces were tangential in the carrot, are distorted after swelling and the originally circular surfaces become elliptical. It has been observed, however, that cylinders from all carrots do not undergo equally the swelling described. Varietal differences may be involved.

The swelling referred to above, however, should not be confused with the subsequent growth of an explant in the presence of coconut-milk. The former is apparently merely vacuolation of cells and a small residual and predetermined ability for growth of cells recently differentiated from the cambium: the latter is the recrudescence of growth in cells which otherwise would not divide again. If explants are removed from close to the cambium the initial swelling, described above, may permit them to increase from 3 to 9 or more mg.: but the subsequent growth may be progressive and potentially unlimited with the proper nutrients and conditions.

Origin of the explant in the carrot. Using a standard period of 14 days it was found that explants removed at successively greater radial distances from the cambium grew less and less. This was the case for explants isolated from the xylem as well as the phloem side of the cambium. Explants which contain the cambium grew well, but were more variable in growth than those which did not include the cambium. Fig. 6 relates the growth obtained in phloem and xylem explants to the radial distance of the explant from the cambium in the intact carrot.

It was also shown that there is a longitudinal gradation in the growth of explants removed at a standard distance from the cambium but at different positions along the axis of the root. The explants from near the root apex grew less than those more remote in the standard time of 14 days. However, the magnitude of this effect was small and the difference along the entire axis of the root was not as great as the gradation in the radial direction from the cambium towards the phloem or the xylem (Fig. 7).

Because explants which contained the cambium gave a relatively high variability in their growth the standard technique was to use only those cylinders with their inner tangential surfaces at 1 mm. from the cambium: these will be referred to as cylinders which are 1 mm. (strictly 1–2 mm.) from the cambium. To minimize the effect of the longitudinal gradation the explants are commonly restricted to a distance between 3 and 7 cm. from the crown. Also, the secondary phloem tissue is preferred to the secondary xylem because the growth of the latter tends to decrease more sharply with distance from the cambium, and it is easier to obtain a larger number of cylinders which will grow from the phloem than from the xylem.

Standard conditions for the growth of carrot-root explants. Where not otherwise explicitly stated the standard conditions used in these experiments may be designated as follows:

The phloem explants (3 mg.) are removed at 1–2 mm. from the cambial surface and grown, usually for 14 days, at 26° C. under the special conditions described in Part I, using 10 ml. of basal medium (White, 1943) supplemented by 15 per cent. by volume of whole coconut-milk.

VARIABILITY AMONG CARROT ROOTS

To test whether conclusions may be drawn from experiments which include explants from more than one carrot, i.e. a mixed, randomized population from many carrots, ten carrots were assayed. Ten standard 3-mg. cylinders were removed from each carrot and were grown under standard conditions for 2 weeks. The results are shown in Fig. 8, in which the data from individual

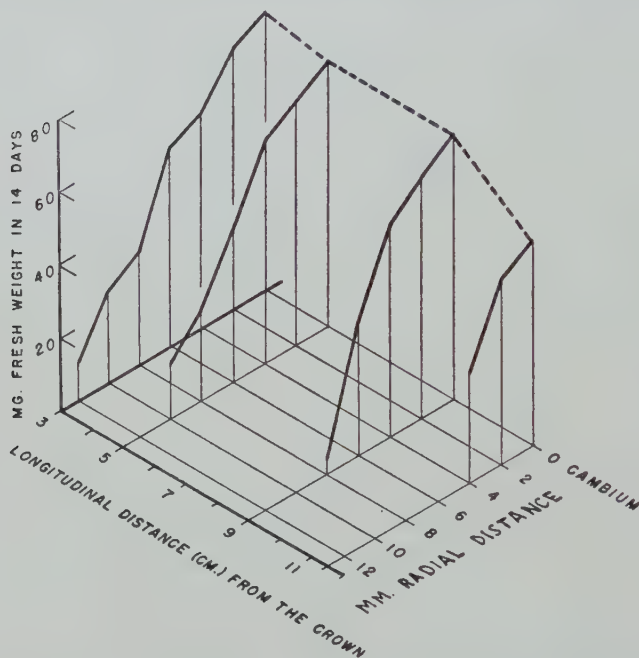


FIG. 7. The effect of removal of carrot-phloem explants (3 mg.) in acropetal succession along the axis of the root.

carrots are arranged according to the mean growth rates of the explants they yielded.

The ability of these explants to grow varied considerably among the different carrots of this stock. The variability in the population of explants from each carrot (as shown by the coefficient of variability) differed from carrot to carrot.

Unfortunately commercially grown varieties of carrot are not genetically homogeneous. The data of Fig. 8 show why one should only draw comparisons between treatments which are tested on a population of explants from the same carrot. (It is well known that the growth of carrot roots is very sensitive to soil conditions, its compactness, &c. One should, therefore, find a stock of carrots which by experience proves to be relatively uniform. Var. 'Long Chantenay' as grown in California has met these requirements.)

A number of varieties of carrots from the same and from different sources have been tested. The differences among the individual carrots within a variety appear to be more important than the differences due to variety *per se*.

VOLUME OF CULTURE MEDIUM

The growth of carrot explants is affected by the volume of the culture solution in the standard rotating culture vessel (Steward, Caplin, and Millar,

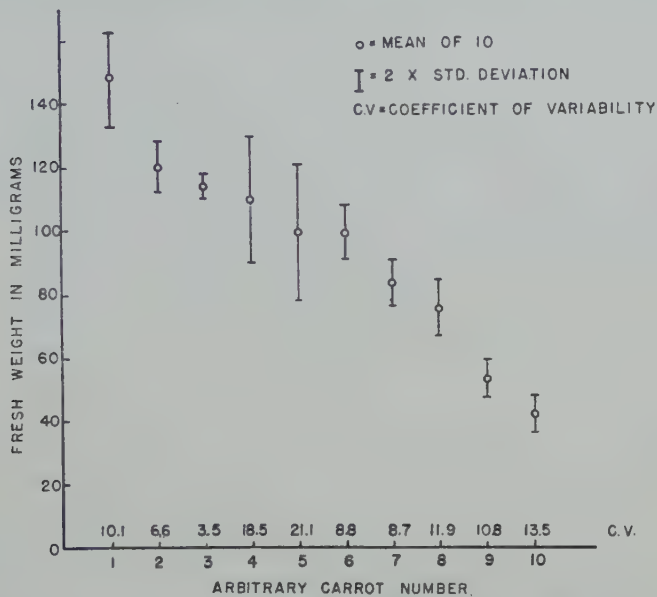


FIG. 8. Variability of phloem explants (3 mg.) from the same and different carrots* as shown by growth during 14 days under standard conditions.

* Variety Long Chantenay (Pieter's Wheeler Seed Co., Calif.).

1951). Fig. 9 shows the effect of volume between 6 and 20 ml. per tube: 10 ml. was the optimal volume for a 10-day growth period. This optimum probably results from a balance between the access to nutrients and the access to oxygen (cf. Steward, Caplin, and Millar, 1951). It appears that from 6 to 10 ml. the increasing access to nutrients permits increasing growth, whereas at volumes of nutrient solution greater than 10 ml. the access to oxygen is progressively reduced by the fractional time of exposure to air.

NUMBER OF TISSUE EXPLANTS PER TUBE

In the exponential phase of growth a single culture is not limited by the constituents of the culture medium. As the culture increases in size and the concentrations of certain nutrient constituents become depleted, the growth-rate should tend to decrease, and to some extent this is true (cf. Fig. 3 of Steward, Caplin, and Millar, 1951). However, even when cultures are transferred to fresh nutrient medium at regular intervals their relative growth

rate still tends to decline, due to the decreases in the ratio of surface to volume.

The following questions now arise: To what extent does the nutrient medium become depleted as the culture grows? How much growth will 10 ml. of nutrient medium support? To answer these questions the number of cultures per tube was increased: Fig. 10 shows the results. The number of explants ranged from 1 to 9 per tube and the cultures were allowed to grow under standard conditions for 17 days. With 1 culture per tube the mean

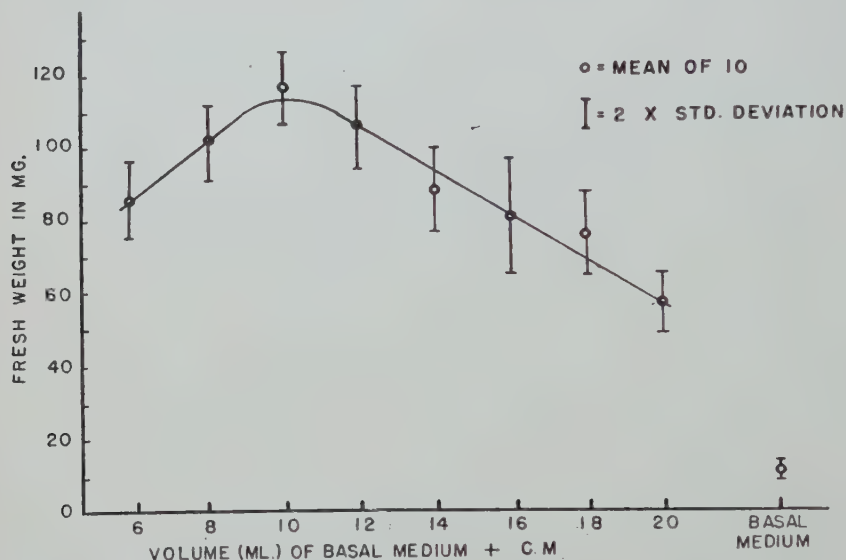


FIG. 9. The effect of the volume of nutrient medium on the growth in 14 days of explants (3 mg.) from carrot root under standard conditions.

weight was 188.4 mg., with 9 explants per tube the mean weight per culture was 75.6 mg. and the total crop per tube was 680.2 mg. Subtracting the initial fresh weight per culture (4.0 mg.), the increase in *total* growth per tube in 17 days by increasing the number of cultures from 1 to 9 per tube was 644.0 mg., and from Fig. 10 it is evident that the capacity of 10 ml. of culture medium to support growth was not yet exhausted.

The increased total growth with the number of cultures in 10 ml. of medium shows that the growth of a single explant cannot be limited by the quantity of nutrients present in 10 ml. of solution. The decrease in the growth-rate of a single culture with time may then be due to the relative decrease in its surface.

Since increasing the number of cultures per tube adversely affects the growth of individual ones (Fig. 10), the question is when does this occur and to what is it due? In another experiment the growth of 1, 2, 3, 5, 6, and 7 cultures per tube was tested at 8, 10, 12, 14, and 15 days. The data are shown in the three-dimensional Fig. 11. The conclusion is that even at 8 days (when

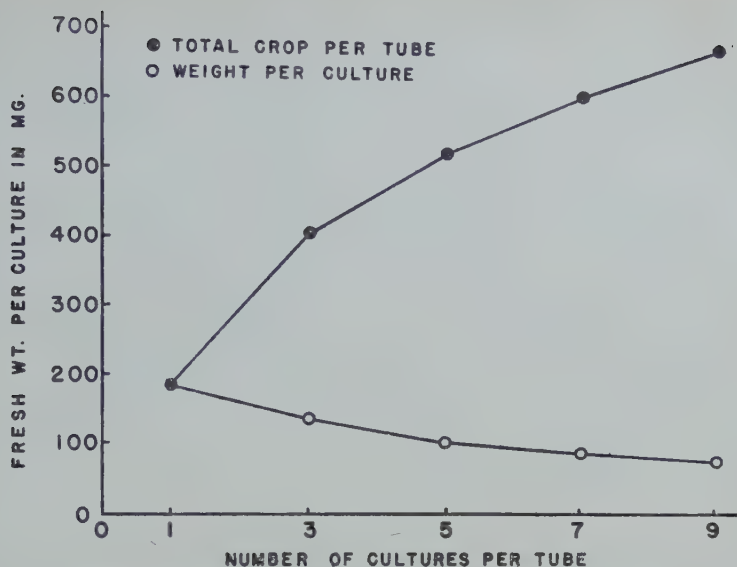


FIG. 10. Effect in 17 days of increasing number of carrot-root explants (4 mg.) per culture vessel.

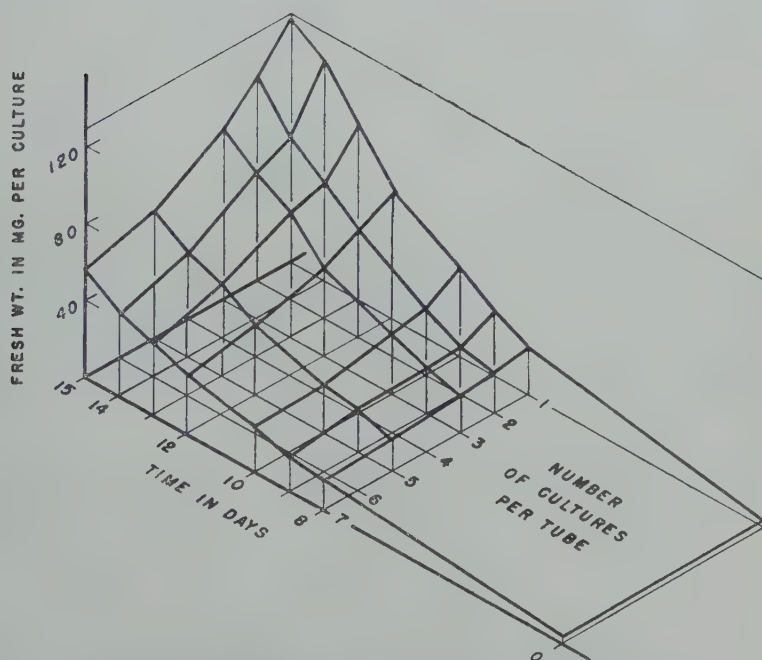


FIG. 11. Effect with time of increasing number of carrot-root explants (3 mg.) per culture vessel.

1 culture in a tube weighed 23.6 mg. and with 2 cultures per tube the mean weight was 20.8 mg. per culture), and very definitely at 9 days (when 1 culture in a tube weighed 32.0 mg.; 2 cultures per tube, 24.0 mg. per culture), the growth of individual cultures is reduced by the presence of more than 1 culture in a tube. The following possible explanations of these observed facts suggest themselves: (1) depletion of a particular nutrient or nutrients, (2) reduced growth due to intermittent physical contact between the cultures, or (3) the production of an inhibitor (or 'staling product') by the growing cultures, such as a metabolic by-product. Of these possibilities the first is discounted by the great total growth which 10 ml. of culture can support (greater than 644 mg.), the second is not considered probable from the observations made while the cultures were revolving. Although only further work can substantiate this hypothesis, the probability is that carrot cultures release metabolites or 'staling products' which tend to reduce the growth of additional cultures in the same batch of medium.

GENERAL CONCLUSIONS

The growth of explants from carrot root under aseptic conditions is subject to many different variables which have been considered. These variables may, however, be standardized yielding a system which may be used for quantitative investigations on the unorganized growth of plant cells and its concomitant physiological processes.

The variable factors which have been considered fall into the following groups:

1. Factors inherent in the supply of an essential growth substance (C.M.F.) for carrot tissue which is found in coconut-milk.
2. Factors inherent in the removal of tissue explants from the carrot root.
3. Factors inherent in the conditions under which the explants are grown.

When all these factors are recognized and wherever possible standardized, the system composed of 3.0 mg. cylindrical phloem explants, cut from carrot root at 1–2 mm. from the cambium, grown under conditions which have been described, in 10 ml. of nutrient medium supplemented by coconut-milk (10 to 15 per cent. by volume), constitutes a most useful tissue culture system for quantitative investigations of growth and its concomitant physiological processes. This is especially so because, lacking the coconut-milk, the growth of such explants is limited so that there is a ready means by which the attributes of cells in the growing and non-growing state may be contrasted.

SUMMARY

1. After a survey of many potential sources of plant tissue cultures, the standard one selected as most suitable for quantitative work consisted of explants from carrot root. The cultures are not subcultured but are established anew on each occasion of their use from carrot roots.

2. The methods and instruments used for the aseptic removal of large numbers of uniform cylinders from carrot root are described, as well as the media suitable for their growth.

3. Data are presented to show that the optimal concentration of coconut-milk in the culture is around 10 to 15 per cent. by volume, though this needs to be determined on every large batch of coconut-milk. The growth factor in coconut-milk is stable to heat; even after 8 hours of continuous autoclaving over half of the activity remains. Low-temperature ($-20^{\circ}\text{C}.$) storage of whole coconut-milk is necessary to preserve its activity.

4. Carrots which are large in diameter and have a circular, clearly defined cambium are the most desirable as a source of explants.

5. As carrot explants are removed at an increasing radial distance from the cambium, whether in the phloem or the xylem, their potentiality for growth decreases in the presence of coconut-milk. The standard conditions developed for this work employ phloem explants removed at 1–2 mm. from the cambium. Although tangential swelling of carrot tissue in water may be demonstrated in the cambial and phloem region, this is not to be confused with the progressive growth and proliferation which is stimulated by coconut-milk.

6. Otherwise comparable carrot explants removed at increasing distance from the crown have a regularly decreasing capacity to grow: the longitudinal gradation, however, is very much smaller than the radial gradient.

7. Within a single variety of carrots from the same source comparable explants from a number of different roots will grow at different rates; for this reason experiments should be made on explants all obtained from the same carrot.

8. The optimum volume of nutrient medium in the standard rotating culture tube is 10 ml. This volume produces the proper balance in the period the cultures are in air and submerged in water.

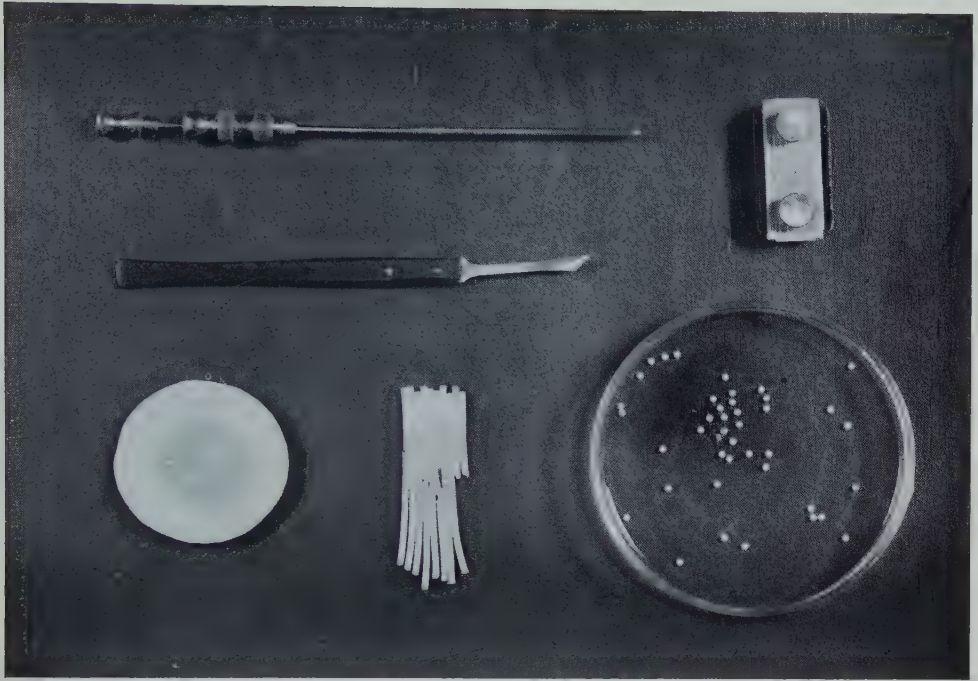
9. By increasing the total number of explants the crop of cultured tissue may be greatly increased, but the weight per tissue culture is strikingly reduced. This effect appears early in the growth and so indicates that the growth of the tissue cultures is reduced by a specific inhibitor, or 'staling product', which originates from the tissue cultures.

ACKNOWLEDGEMENTS

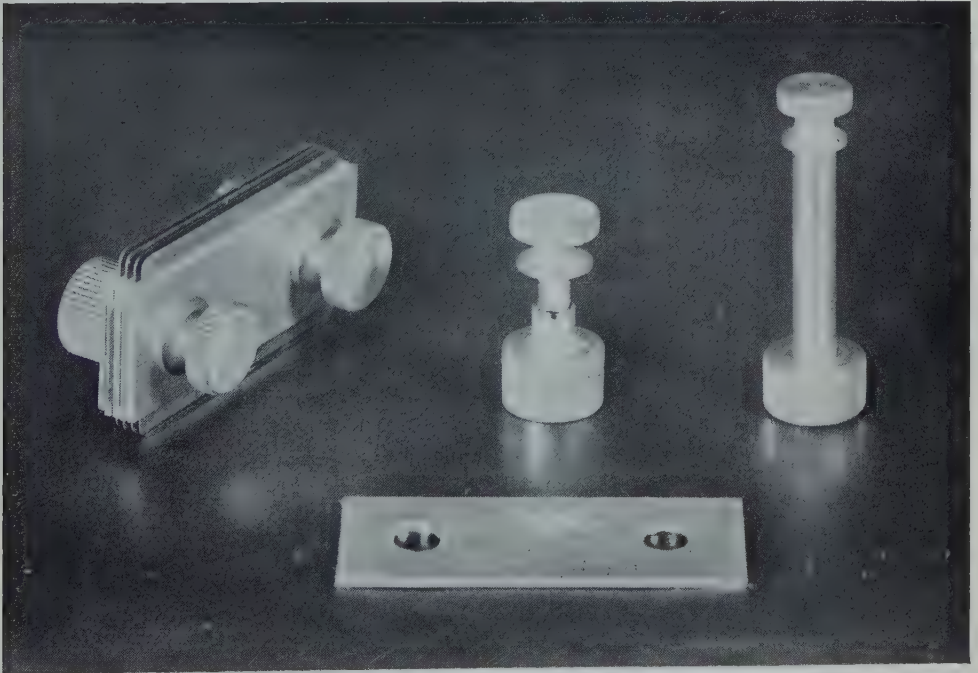
These investigations have been supported by grants to one of us (F. C. S.) from the Committee on Growth of the National Research Council and from the National Cancer Institute, National Institutes of Health, U.S. Public Health Service. Miss Mary Case acted as research assistant during much of the work and carried out manipulations requiring aseptic precautions. During part of the work one of us (S. M. C.) held a Post-doctoral Fellowship of the National Institute of Health. For all this help we express our gratitude.

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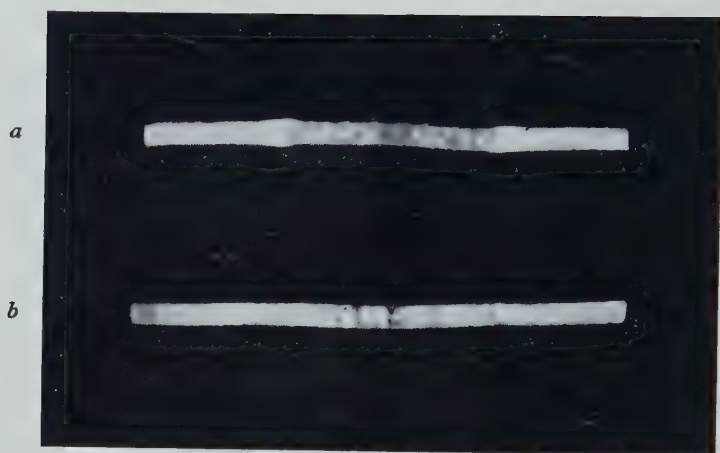
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(a)



(b)



Studies in the Physiology of Parasitism

XVII. Enzyme Secretion by Strains of *Bacterium carotovorum* and other Pathogens in relation to Parasitic Vigour

BY

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With three Figures in the Text

ABSTRACT

1. When tested on potato tubers and roots of turnip, swede, and carrot, the organisms *Bacterium aroideae*, *B. carotovorum* (i), *B. carotovorum* (ii), and *Phytoplasma carotae* show diminishing pathogenicity in the order named. Attack on potato is greatest at 25–35° C., and falls off gradually from 20° to 10°, below which it ceases.

2. The susceptibility of potato tubers rises as they grow to full size but falls during the dormant period. With the active parasite *B. aroideae* there is a considerable rise in susceptibility as the tuber reaches the stage of sprouting.

3. Susceptibility of potato tubers is increased by storage for some time at an elevated temperature (c. 35° C.), or by raising their water-content, either by soaking or by direct injection, and especially by the latter. Similar responses to these treatments are given by enzymic preparations of the bacteria.

4. The greater pathogenicity of *B. aroideae* than of the *carotovorum* strains is associated with a greater rate of multiplication of the former under comparable conditions and with a greater tendency of the latter to an acid-forming type of metabolism which is unfavourable to multiplication of the organism and to the activity of its enzyme.

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A. INTRODUCTION

THE work described here is a continuation, along similar lines, of that of Fernando and Stevenson (1951) published as the preceding number of this series. The organisms used were limited to bacteria, but on the other

hand the host material was extended to a variety of vegetable tissues and to potato tubers at various stages of development.

B. MATERIALS AND METHODS

The organisms used were

1. *Bacterium aroideae*, strain 66 D of Dr. W. J. Dowson's collection.
2. *B. carotovorum* (i), isolated from broccoli in 1929.
3. *B. carotovorum* (ii), originally isolated at Cheshunt from tomato.
4. Isolate from carrot.

The first three were stock cultures maintained in the Bacteriological Laboratory; the fourth showed good agreement in its cultural behaviour with *Phytophthora carotae*, described by Kendrick (1934) as pathogenic to leaves and roots of carrot. It will be referred to as such throughout this paper. All the cultures were kept on potato agar slopes incubated at 25° C.

Potatoes and other vegetables, after surface sterilization in 0.2 per cent. mercuric chloride and subsequent washing, were inoculated in either of two ways. The method generally adopted was that described by Granger and Horne (1924) which consists in removing a cylindrical plug of tissue of standard depth (1.5 cm.) by a small corkborer (No. 2). After this, 0.1 c.c. of the bacterial suspension was pipetted into the cavity and the opening bridged over with a mixture of paraffin wash and vaseline. The plug of tissue was not replaced, as it was found that by this means more uniform results were obtained in cases where the amount of attack was slight, the plug when replaced sometimes escaping attack altogether. Wherever possible, comparative inoculations were made by placing two or more of the bacterial isolates on the same tuber (or root), thereby largely eliminating the error due to variation of resistance from one individual to another.

In the second method, cylinders of tissue approximately 4 cm. long and 1.8 cm. wide were cut with a No. 12 corkborer, and after surface sterilization in the usual way (a process which involves some uptake of water), a cavity of 1 cm. depth was prepared at one end of the cylinder by means of a No. 2 corkborer. The bacterial suspension was placed in this cavity.

Tissues inoculated by either method were incubated at 25° in a saturated atmosphere, and after a few (2-4) days the amount of attack was determined by scraping off and weighing the rotted tissue.

For purposes of preparing enzymic solutions from the bacteria under examination, plant decoctions of two strengths were made up as follows:

- (a) Dilute, referred to as *vegetable extract*. Two hundred grammes of potato or 300 g. of turnip or carrot was cut into small pieces and boiled until soft. The cooked tissue was squeezed through cloth, the liquid made up to a litre with distilled water, and autoclaved.
- (b) Concentrated, referred to as *vegetable juice*. The raw tissue was finely minced and extracted under pressure. The liquid was then autoclaved, centrifuged to remove precipitate, and again autoclaved.

The nutrient media were placed in ordinary-sized medicine bottles, 20 c.c. to each, and after sterilization inoculated in a uniform manner with a bacterial suspension. The bottles were laid on their sides to give a shallow layer of liquid and incubated at 25° C.

For enzymic tests the culture solutions were either used as such, i.e. as crude external enzyme, or they were partially purified by precipitation with 4 volumes of 95 per cent. alcohol. The precipitate was dried over calcium chloride and finally ground with its weight of silver sand. This powder was used at the rate of 0.5 g. to 50 c.c. water.

Enzymic activity was usually measured by determining the time (reaction time, R.T.) required for the maceration of tissue discs 0.5 mm. thick. In any one experiment the test discs were taken from the medullary tissue of the same tuber.

In some experiments cylinders of tissue with a prepared cavity at one end (as described on p. 236) were used. A small quantity, 0.1 c.c. of the enzymic solution, was placed in the cavity, and after 24 hours' incubation at 25° the amount of rotted tissue was determined. This mode of testing gives a closer approximation to the conditions under which a bacterial inoculum attacks the tissue.

Estimations of pH were made with the Universal Buffer Solution prepared by British Drug Houses according to the formula prescribed by Prideaux and Ward (1924).

C. PATHOGENICITY OF ISOLATES

All four isolates were inoculated by the Granger and Horne method into mature tubers of seven varieties of potato. Table I gives the average amount of rotting (g.) produced after 3 days' incubation at 25° C. For each determination 10 tubers were used.

TABLE I
Pathogenicity of Isolates on Potato Varieties

Variety.	<i>B. aroideae</i> .	<i>B. carot.</i> (i).	<i>B. carot.</i> (ii).	<i>P. carotae</i> .
Majestic . .	5.4 ± 1.1	1.7 ± 0.23	0.82 ± 0.01	0.21 ± 0.002
" . .	3.4 ± 0.6	0.95 ± 0.13	0.54 ± 0.15	0.22 ± 0.02
" . .	6.6 ± 0.8	1.25 ± 0.22	0.87 ± 0.24	0.22 ± 0.03
King Edward . .	2.1 ± 0.34	0.52 ± 0.06	0.26 ± 0.05	0.14 ± 0.05
" . .	2.5 ± 0.15	0.71 ± 0.1	0.39 ± 0.09	0.11 ± 0.03
Gladstone . .	4.55 ± 0.64	0.53 ± 0.13	0.31 ± 0.06	—
Doon Star . .	6.15 ± 0.29	2.2 ± 0.12	0.64 ± 0.29	0.17 ± 0.004
Great Scot . .	1.52 ± 0.09	0.44 ± 0.06	0.25 ± 0.03	—
Epicure . .	2.8 ± 0.4	0.27 ± 0.02	0.19 ± 0.02	—
Arran Pilot . .	1.4 ± 0.25	0.37 ± 0.05	0.2 ± 0.03	—

All ten experiments clearly show that the pathogenicity of the isolates diminishes in the order *B. aroideae*, *B. carotovorum* (i), *B. carotovorum* (ii), and *P. carotae*. It was further noted that at the time the final readings were taken, attack by *B. aroideae* and *B. carotovorum* (i) appeared to be still

progressing whereas the tissue rotted by the other two bacteria had already begun to shrivel up.

Table II gives a comparison (with tubers of var. 'Majestic') of the two methods of inoculation, viz. the Granger and Horne method and the hollow-cylinder method already described. The experiment was run for 48 hours.

TABLE II

Effect of Method of Inoculation (var. 'Majestic')

Method.	<i>B. aroideae</i> .	<i>B. carot.</i> (i).	<i>P. carotae</i> .
Granger and Horne .	2.25±0.09	1.03±0.01	0.29±0.04
Cylinder	3.24±0.14	2.09±0.14	1.05±0.1

The differential behaviour of the isolates is again shown. It is also clear that the second method of inoculation gives greater attack than the first, and this is especially true for the weak parasite *P. carotae*. Undoubtedly the increased parasitism shown by the cylinders is to be set down to their having a higher water-content as a consequence of the method of their preparation. The effect of water-content on amount of attack will be dealt with in a later section.

Three experiments were carried out with other vegetables with the results shown in Table III.

TABLE III

Host.	<i>B. aroideae</i> .	<i>B. carot.</i> (i).	<i>B. carot.</i> (ii).	<i>P. carotae</i> .
Turnip . . .	2.88±1.37	0.74±0.62	0.3 ±0.13	trace
Swede . . .	1.36±0.32	0.19±0.07	trace	0
Carrot . . .	4.98±0.33	0.98±0.09	0.18±0.04	0

The standard errors, with turnip as host, are high on account of the high susceptibility of a few individuals in the batch tested. Nevertheless it is clear that the order of pathogenicity is as before. With carrot, attack was mainly concentrated on the stele, the wide cortical zone being left almost intact. This differential effect was also noted by Jones (1900), who suggested that it was due to a higher water-content of the stelar region. The later work of Buston and Kirkpatrick (1931), however, failed to establish any difference between stele and cortex in the matter of water content or of chemical composition or of distribution of pectin.

The foregoing experiments show that the order of pathogenicity of the isolates is the same on all the four types of vegetable tissue used.

D. EFFECT OF TEMPERATURE ON RATE OF ATTACK

Potatoes of var. 'Majestic' were inoculated with the various bacteria and incubated for 3 days at temperatures over the range 10–35° C. Before inocula-

tion they were stored for a few hours at the various temperatures. The results are shown in Fig. 1.

In all cases the greatest amount of rotted tissue was produced at 25–35° with a maximum probably a little above 25°. There was a sharp rise in activity

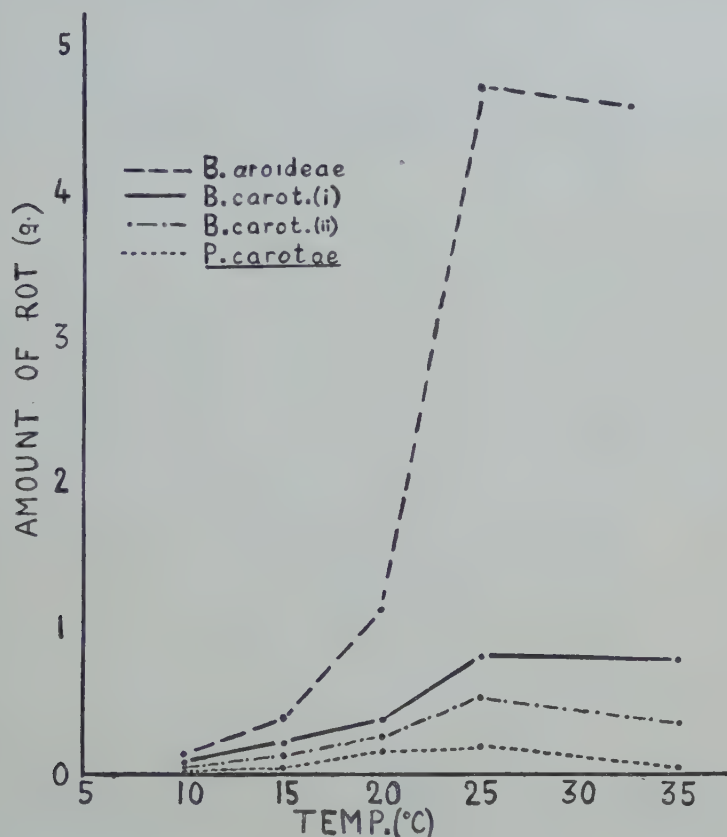


FIG. 1. Illustrating effect of temperature on rate of attack of potato-tuber tissue by *B. aroideae*, *B. carotovorum* (i), *B. carotovorum* (ii), and *P. carotae*.

between 20° and 25°, especially marked for the active parasite *B. aroideae*. Below 10° the amount of attack was slight.

Somewhat similar results were recorded by Brierley (1928), who states that *B. carotovorum* gave little attack outside the range 21–29°, and that *B. aroideae* was particularly active from 20° upwards, with a maximum as high as 33.5°. Recently Rudd-Jones and Dowson (1950) have determined the temperature relationships of *B. carotovorum* as a parasite of potatoes in storage and have correlated resistance at low temperatures with the formation of an effective cork barrier under those conditions.

E. EFFECT OF MATURITY OF POTATO TUBERS ON RATE OF ATTACK

Tubers of the varieties 'King Edward', 'Gladstone', 'Arran Banner', 'Great Scot', 'Epicure', and 'Arran Pilot' were inoculated with *B. aroideae*, *B. carot.* (i), and *B. carot.* (ii) at four stages in their development, viz. (a) in July when the tubers were small, (b) in September when the crops were harvested, (c) in January, after storage in the interval in a cool cellar, and (d) in May when the tubers had begun to sprout. The inoculations were made in 10-plicate, and the tubers so treated were incubated at 25° C. for 3 days. The average amounts of attack are set out in Table IV.

TABLE IV

Amounts of Attack on Potatoes at different Stages of Maturity

Isolate.	Date of inoculation.	King Edward.	Gladstone.	Arran Banner.	Great Scot.	Epicure.	Arran Pilot.
<i>B. aroideae</i>	July	1.41	1.88	2.34	1.83	2.68	2.07
	Sept.	1.76	2.52	2.49	1.41	2.85	2.17
	Jan.	2.33	1.9	1.19	1.17	0.61	—*
	May	6.05	4.55	3.59	1.52	2.77	1.41
<i>B. carot.</i> (i)	July	0.48	0.77	0.66	1.17	0.66	0.59
	Sept.	0.99	1.25	0.97	0.86	1.45	0.95
	Jan.	0.79	0.59	0.49	0.31	0.29	—*
	May	0.95	0.53	0.48	0.44	0.27	0.37
<i>B. carot.</i> (ii)	July	0.35	0.53	0.4	0.29	0.4	0.47
	Sept.	0.6	0.81	0.59	0.41	1.05	0.79
	Jan.	0.42	0.28	0.34	0.19	0.17	—*
	May	0.38	0.31	0.46	0.25	0.18	0.2

* Stock of material insufficient for all the tests.

The standard errors of the data are not included in Table IV but they were calculated and they furnish the basis on which Table V is constructed. The degree of significance of the differences between July–September, September–January, and January–May is set out in the table.

The results of Tables IV and V may be summarized as follows:

1. In every instance the same order of pathogenicity was maintained, viz. *B. aroideae* > *B. carot.* (i) > *B. carot.* (ii) (Table IV).
2. In 16 out of 18 comparisons there was more attack with the September than with the July tubers. Both the exceptions were with the 'Great Scot' variety. The differences shown had the highest degree of significance with the two weak parasites.
3. With one exception the increase of susceptibility in September was followed by a sharp decrease in the January tubers.
4. Sprouting of the tubers increased their susceptibility to the vigorous parasite *B. aroideae*. The effect was specially well marked with the three

late varieties (the first three in the tables). With the two weak parasites, any effect of sprouting was slight and variable.

5. There were no substantial differences in susceptibility between the six varieties tested.

TABLE V

Significance of Differences in Seasonal Amount of Attack

Isolate.	Com- parison.	King Edward.	Glad- stone.	Arran Banner.	Great Scot.	Epi- cure.	Arran Pilot.
<i>B. aroideae</i>	July < Sept.	N.S.	S.	N.S.	Neg. N.S.	N.S.	N.S.
	Sept. > Jan.	Neg. N.S.	S.	H.S.	N.S.	H.S.	—
	Jan. < May	H.S.	H.S.	H.S.	N.S.	H.S.	—
<i>B. carot.</i> (i)	July < Sept.	H.S.	H.S.	H.S.	Neg. N.S.	H.S.	H.S.
	Sept. > Jan.	N.S.	H.S.	H.S.	H.S.	S.	—
	Jan. < May	N.S.	Neg. N.S.	Neg. N.S.	N.S.	Neg. N.S.	—
<i>B. carot.</i> (ii)	July < Sept.	H.S.	N.S.	H.S.	H.S.	H.S.	H.S.
	Sept. > Jan.	H.S.	H.S.	H.S.	H.S.	H.S.	—
	Jan. < May	Neg. N.S.	N.S.	S.	N.S.	N.S.	—

N.S. = non-significant, $P > 0.05$

S. = significant, $P, 0.05-0.01$

H.S. = highly significant, $P < 0.01$.

Neg. = negligible.

The fall in susceptibility between September and January may be due, in whole or in part, to lowered water-content resulting from dry storage. Sprouting, however, further reduces water-content of the tubers, especially with the vigorously sprouting early varieties. The fact that tubers on sprouting become more susceptible to *B. aroideae* indicates that there is an inherent increase in susceptibility which more than counteracts the effect of lowered water-content. With the weaker parasites these two opposite tendencies cancel each other out.

The overall seasonal drift of susceptibility is shown in Fig. 2, in which the data for all six varieties have been averaged, for each of the three isolates.

F. EFFECT OF WARM PRE-STORAGE ON RATE OF ATTACK

Vasudeva (1930) found that when apples had been stored for some time at 30–35° they became susceptible to attack by *Botrytis allii*, which had no such effect on normal fruits. Chona (1932) showed that a similar treatment much intensified the attack by *Fusarium fructigenum* on apple fruit and also rendered the latter susceptible to *F. coeruleum*. More recently Fernando and Stevenson (1950) have shown a similar response of potato tubers to *Bacillus subtilis*. These observations are extended in the present section, chiefly for the potato varieties 'King Edward' and 'Majestic'.

Batches of 10 tubers were stored at 35° for various periods, the controls remaining meanwhile at laboratory temperature. Before inoculation the pre-heated tubers were allowed to cool for 3 hours, after which all the batches were inoculated and incubated at 25° for 4 days. The data for the potato variety 'Majestic' are set out in Table VI, in which also the significance (*P*) of the differences recorded is included.

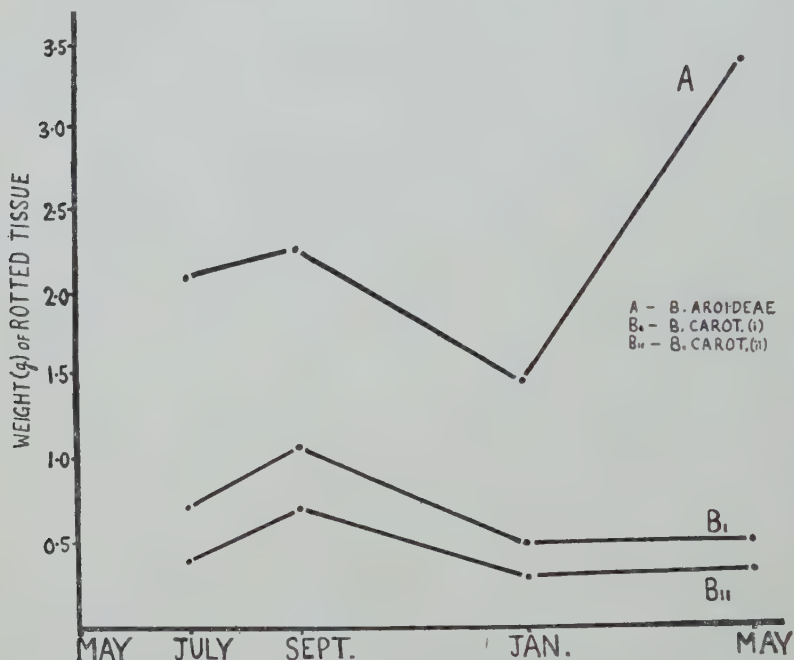


FIG. 2. Illustrating seasonal drift of susceptibility to attack by three bacterial pathogens.

A study of the *P* columns shows that the significance of the differences in amount of attack which are brought about by heat pretreatment does not increase regularly with duration of the treatment. This is due without doubt to the variation between samples which leads to variability in the control figures and to the variation in susceptibility of individual tubers in the batch, this variation affecting the magnitude of the difference which reaches significance. A significant difference, when there were no grounds for expecting any such difference, was got in one case, viz. with *B. carot.* (i), after 2 days' pretreatment. This difference was in favour of the controls and could arise only from sampling error. Nevertheless the trend of the figures in Table VI is unmistakable. From the 10th day onwards the treated tubers show in all cases a considerably greater attack than the controls, though the difference is in itself significant only in about half of the comparisons. A parallel series with var. 'King Edward' showed the same general behaviour.

TABLE VI
Increase of Susceptibility of Potato Tubers with Duration of Heat Pretreatment

Pre-treatment time (days).	Treatment.	<i>B. aroideae</i> .		<i>B. carot.</i> (i).		<i>B. carot.</i> (ii).		<i>P. carotae</i> .	
		Av. rot-ting.	P	Av. rot-ting.	P	Av. rot-ting.	P	Av. rot-ting.	P
2	Heated	3.51	—	0.61	Neg.	0.47	—	0.21	—
	Control	3.44		0.95		0.54		0.22	
5	Heated	9.18	0.05	1.02	Neg.	0.73	—	0.26	—
	Control	6.55		1.27		0.87		0.22	
7	Heated	8.37	0.05	0.76	—	0.54	—	0.21	—
	Control	4.6		0.78		0.46		0.18	
10	Heated	9.39	0.3	1.46	0.2	0.9	0.05	0.29	0.2
	Control	6.61		0.89		0.43		0.15	
14	Heated	9.23	< 0.01	1.27	< 0.01	1.16	0.1	0.3	0.01
	Control	5.36		0.6		0.4		0.17	
21	Heated	8.32	0.02	1.36	< 0.01	0.94	0.2	0.6	0.05
	Control	4.46		0.63		0.59		0.14	

The large amount of incubator space which would have been required precluded the carrying out of similar experiments with turnips or swedes, but carrots were tested in this respect. With this host, however, the period of treatment could not be extended beyond 6 days without damage to the tissue generally. In the two experiments carried out, with the usual amount of replication, the average amount of attack by *B. aroideae* was approximately doubled by heat pretreatment, but the difference in either case was barely significant at the 0.05 level.

The greater susceptibility of potato tubers consequent on preheating does not persist, to judge from certain experiments in which it was found that after 6 days' storage at laboratory temperature preheated tubers were no longer distinguishable in susceptibility from the controls. To establish this result, however, more extensive experiments are required.

The effect of heat pretreatment in intensifying bacterial invasion is paralleled by the behaviour of bacterial enzymic preparations, as shown in Table VII,

TABLE VII
Effect of Heat Pretreatment on Rate of Attack by Pectinase Enzyme

Days of pre-treatment.	Treatment.	<i>B. aroideae</i> .		<i>B. carot.</i> (i).		<i>B. carot.</i> (ii).		<i>P. carotae</i> .	
		Av. rot-ting.	P	Av. rot-ting.	P	Av. rot-ting.	P	Av. rot-ting.	P
6	Heated	1.25	0.02	0.8	0.2	0.49	0.1	1.07	0.4
	Control	0.8		0.62		0.35		0.8	
10	Heated	2.66	< 0.01	1.82	< 0.01	0.93	< 0.01	2.07	< 0.01
	Control	0.84		0.94		0.53		1.06	

which records a typical experiment in this connexion. Cylinders were cut from preheated (35°) and control potato tubers (var. 'Majestic') and 0.1 c.c. of purified enzyme placed on each in the manner already described. Each reading in the table gives the average weight of rotted tissue from 10 cylinders after 24 hours' incubation.

The greater susceptibility of preheated tubers to enzymic action is highly significant after 10 days of the treatment. These results suggest that the treatment increases susceptibility to bacterial attack either by changing the composition of the middle lamella substance in such a way as to make it more readily hydrolysed, or by lessening the effect of some factor which slows down enzyme action. No differences in the middle lamella could be detected when sections from heated and control tissues were examined microscopically after staining with ruthenium red.

It will be noted that the rate of attack by enzymic preparations of the four bacteria does not fall into line with the order of pathogenicity of the bacteria themselves. In particular the enzymic preparation of *P. carotae*, the least pathogenic of the isolates, is comparable in activity with that of *B. aroideae*, the most active. This subject will be taken up in a later section.

G. EFFECT OF WATER-CONTENT OF HOST TISSUE ON RATE OF ATTACK

Cylinders of host tissue with a cavity at one end, as described on p. 236, were used for this work. These were prepared in the following three ways:

- (a) *Control*, rapidly washed in sterile water to remove contents of broken cells from the surface.
- (b) *Soaked*, as in (a) but also kept submerged in sterile water for 6 hours to take up water.
- (c) *Injected*, as in (a) but then injected with water by evacuation under a filter-pump.

After the various treatments all cylinders were placed in closed humid containers, inoculated each with 0.1 c.c. of bacterial suspension and incubated at 25° C. for 1–2 days. Three cylinders from each batch were used for water-content determinations. Table VIII gives the results of two such experiments, the amounts of rotting given being the averages from 10 tubers (var. 'Majestic').

TABLE VIII

Effect of Water-content on Amount of Rotting

Duration of expt.	Bacterium.	Control.		Soaked.		Injected.	
		Rotting.	% water.	Rotting.	% water.	Rotting.	% water.
1 day	<i>B. aroideae</i>	1.22 ± 0.08	80.5	2.16 ± 0.23	82.7	7.07 ± 0.26	82.6
	<i>B. carot. (i)</i>	0.87 ± 0.04		1.85 ± 0.15		4.48 ± 0.32	
	<i>P. carotae</i>	0.15 ± 0.03		0.56 ± 0.06		1.06 ± 0.14	
2 days	<i>B. aroideae</i>	3.24 ± 0.14	83.6	4.9 ± 0.19	85.5	7.1 ± 0.2	85.6
	<i>B. carot. (i)</i>	2.1 ± 0.14		3.66 ± 0.16		7.4 ± 0.17	
	<i>P. carotae</i>	1.05 ± 0.1		2.3 ± 0.27		5.2 ± 0.19	

All the differences shown are significant at more than the 0.01 level. The striking feature in all cases is that, whereas the soaking treatment clearly increased susceptibility to attack, the effect of this was small compared with that of injection. The water-content of soaked or injected cylinders was about 2 per cent. higher than that of the controls, and while the water-content after injection was usually somewhat higher (in the various experiments made in this connexion) than after mere soaking, the difference was always small and often negligible. After either soaking or injection one must presume that the suction pressure of the cells is reduced to approximately zero. It is suggested therefore that some factor other than turgidity underlies the greater susceptibility of cylinders which have been injected.

In view of the possibility that the process of injection might be injurious to the tissue, a subsidiary experiment was carried out in which the period of treatment under the pump was varied between the limits of $\frac{1}{4}$ hour and $1\frac{1}{2}$ hours. Cylinders so treated showed the usual great increase of susceptibility as compared with the controls, but there was no suggestion that the effect was increased by a longer period of treatment.

The effect of soaking or injection of tissue on susceptibility to bacterial attack is reproduced with enzymic preparations made from these. Table IX gives the results of such a comparison, the figures being the amounts of attack in 24 hours by the purified enzymes. Table X records another experiment of the same kind in which *B. aroideae* is compared with an enzymic preparation from it.

TABLE IX

Effect of Water-content on Amount of Enzymic Attack

Enzyme.	Control.	Soaked.	Injected.
<i>B. aroideae</i> . . .	0.58 ± 0.16	0.86 ± 0.09	1.87 ± 0.17
<i>B. carot.</i> (i) . . .	0.9 ± 0.05	1.42 ± 0.12	2.66 ± 0.21
<i>B. carot.</i> (ii) . . .	0.3 ± 0.04	0.83 ± 0.06	0.91 ± 0.07
<i>P. carotae</i> . . .	0.73 ± 0.08	1.74 ± 0.16	2.89 ± 0.24

Water-content: Control, 81.1%; Soaked, 82.6%; Injected, 83.9%.

TABLE X

Effect of Water-content on Attack by B. aroideae and its Enzyme

	Control.	Soaked.	Injected.
Bacterium . . .	1.05 ± 0.06	1.3 ± 0.15	4.26 ± 0.47
Enzyme . . .	0.47 ± 0.05	0.51 ± 0.04	2.67 ± 0.26

Water-content: Control, 80.8%; Soaked, 84.2%; Injected, 83.9%.

The differences due to treatments follow the same course as with the bacteria themselves. All those shown in Table IX are highly significant, apart from two (*B. aroideae*, control v. soaked; and *B. carot.* (ii), soaked v. injected). Similar results are described by Fernando and Stevenson (1950)

using the internal enzyme of *Botrytis cinerea* and the external enzyme of *B. carotovorum*.

One may assume that the effect of soaking, or of injection, would be to facilitate the diffusion of enzyme into the tissue. But inasmuch as the water-content of injected and soaked tissues is often very similar, and as these treatments give rise to markedly different degrees of susceptibility, it is difficult to accept the view that rate of diffusion is the only factor concerned. It is possible that the process of injection washes out some substance which slows down enzymic action.

Similar experiments on a smaller scale were carried out with turnip, swede, and carrot. The overall indication was that injection increased susceptibility for all tissues and bacteria used. The effect of mere soaking was not so certain, but the tendency was usually in the same direction, though often not so pronounced as to be statistically significant.

H. ANALYSIS OF DIFFERENTIAL PATHOGENICITY OF ISOLATES

Throughout this work it was found that, though the parasitic activity of the bacteria concerned could be altered by changes in the condition of the host tissue, the comparative differences in the rate of attack always remained the same. *B. aroideae* was under all conditions the most active and the remaining three followed in the order *B. carotovorum* (i), *B. carotovorum* (ii), and *Pseudomonas carotae*. Experiments were undertaken with the idea of separating out the factors underlying these differences in pathogenicity. These have been rather of an exploratory nature, and more intensive work, probably with more refined techniques, will be required before the various factors can be considered to be established. The results will therefore be given only in summary form.

So far as can be seen at present there is no outstanding difference in the properties of the pectinase enzymes prepared from each of the four bacteria. Thus, whereas Fernando (1937) showed that the enzymes of *Botrytis cinerea* and *Bact. carotovorum* have optima at approximately pH 6 and 8 respectively, the bacterial enzymes of the present work all agreed in being most active at pH 8. The curves of activity are set out in Fig. 3. These preparations were made from cultures on potato extract and partially purified by one precipitation with alcohol. The general shape of the curves is the same for all, and the only suggestion of a difference is the gentler slope towards the acid side of the curves for the two strains of *B. carotovorum*.

Fig. 3 illustrates a result which has been repeatedly met with in enzymic studies of these bacteria, viz. that under comparable conditions the preparation from *B. aroideae* is the most active. Those of the *B. carotovorum* strains are distinctly weaker, and in the order *B. carotovorum* (i) followed by *B. carotovorum* (ii). This is also the order of their pathogenicity, but the enzyme of *P. carotae* does not obviously fall into this scheme, as this organism is the weakest of all four tested, and in many of the tests its enzyme comes next to

that of *B. aroideae* in activity. These behaviours will now be further illustrated and to a certain extent analysed.

The greater activity of *B. aroideae*, both as regards pathogenicity and enzyme-production, over the two strains of *B. carotovorum* would appear to rest upon the following differences:

1. *B. aroideae* shows a greater multiplication rate on a variety of media.

This is illustrated in Table XI, which gives the numbers of bacteria

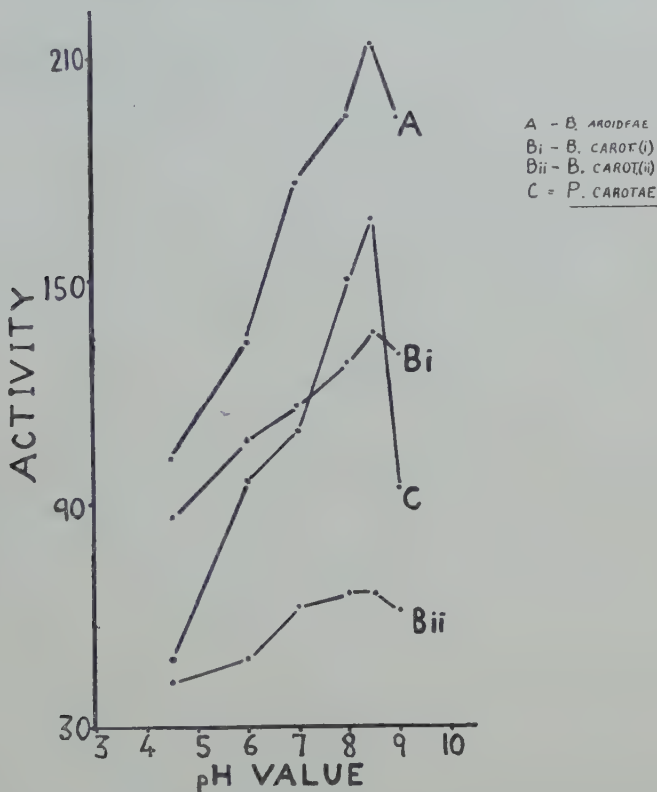


FIG. 3. Illustrating effect of pH value on the activity of pectinase enzymes of *B. aroideae*, *B. carotovorum* (i), *B. carotovorum* (ii), and *P. carotae*.

present per c.c. after 24 hours' culture on a weaker (potato extract) and a stronger (potato juice) medium and on a synthetic medium which is approximately equivalent to the former.

2. Both strains of *B. carotovorum* show a greater tendency to acid formation than does *B. aroideae*. This effect has been verified on such media as potato, turnip, and carrot extracts, potato extracts buffered or unbuffered at various initial pH's, and on carrot extract reinforced by addition of asparagin, ammonium chloride, or glucose. Tables XII–XIV illustrate these results.

TABLE XI

Effect of Medium on Multiplication of B. aroideae and B. carotovorum (i)

Medium.	Bacterial numbers ($\div 10^6$).	
	<i>B. aroideae</i> .	<i>B. carot.</i> (i).
Potato Extract	43	5
Potato Juice	361	23
Brown's Synthetic Medium	39	7

Table XII gives a comparison of *B. aroideae* and *B. carotovorum* (i) on potato extract (weak) and potato juice (undiluted). The tendency of the former organism to swing the reaction to an alkaline point is shown, as also the tendency of *B. carotovorum* (i) to maintain an acid reaction on the richer medium. This leads to poor growth and low enzyme activity.

TABLE XII

Comparison of B. aroideae and B. carotovorum (i) on Potato Media.
(Measurements after three days' growth)

Bacterium.	Potato extract (weak) (initial pH, 6.9).			Potato juice (undil.) (initial pH, 5.6).		
	pH.	R.T.* (hrs.).	Growth.	pH.	R.T. (hrs.).	Growth.
<i>B. aroideae</i>	8.3	0.2	Strong	8.0	0.15	Strong
<i>B. carot.</i> (i)	8.0	0.55	Strong	4.8	2.7	Weak

* R.T. = Reaction time (v. p. 237).

On turnip or carrot media *B. aroideae* slowly brings the reaction to an alkaline point, whereas the *B. carotovorum* strains give no perceptible drift in that direction. Under these conditions enzymic activity of the latter is very weak.

Table XIII shows the effect of various additions to carrot extract on pH drift and enzymic activity, after 2, 6, and 14 days' growth.

TABLE XIII

pH Changes and Enzymatic Activity by B. aroideae and B. carotovorum (i)
on Modifications of Carrot Extract

Nutrient.	2 days.		6 days.		14 days.	
	pH.	R.T. (hrs.).	pH.	R.T. (hrs.).	pH.	R.T. (hrs.).
<i>B. aroideae</i>						
Carrot extract	5.9	2.2	5.9	2.3	8.0	1.25
„ +0.5% asparagin . .	6.2	0.6	8.5	0.33	8.4	0.3
„ +0.5% NH ₄ Cl . . .	5.5	3.25	5.8	12	5.6	24
„ +0.5% glucose . . .	5.4	4.7	5.5	3.25	7.4	2.7
<i>B. carotovorum</i> (i)						
Carrot extract	5.0	> 24	4.8	> 24	4.8	> 24
„ +0.5% asparagin . .	5.0	„	5.2	„	5.0	„
„ +0.5% NH ₄ Cl . . .	4.8	„	4.6	„	4.6	„
„ +0.5% glucose . . .	4.9	„	4.9	„	4.7	„

The following points are to be noted:

1. *B. aroideae* shows a greater tendency to develop an alkaline reaction. This is intensified by addition of asparagin to the culture medium, but slowed down by addition of glucose and especially of ammonium chloride, which causes the reaction to remain acid throughout. An acid reaction is developed by *B. carotovorum* cultures on all the media.
2. Enzymatic activity of *B. aroideae* is greater the more quickly the reaction moves to an alkaline point. Thus it is weakest in the medium with ammonium chloride added, in which medium the enzyme becomes progressively deactivated with time. No appreciable amount of enzyme was detected in any of the *B. carotovorum* cultures.

Table XIV gives the behaviour of *B. aroideae* and *B. carotovorum* (i) on comparable extracts prepared from the cortical and stelar regions of carrot roots.

In addition to the usual differences between *B. aroideae* and *B. carotovorum* (i), it is clear that alkaline drift and enzymic activity are greater on extracts of stele than of cortex. These results show close agreement with the behaviour (p. 238) of the organisms on stele and cortex of carrot roots.

TABLE XIV

pH Changes and Enzymic Activity by B. aroideae and B. carotovorum (i) on Cortical and Stellar Extracts of Carrot

Bacterium.	Extract.	3 days.		9 days.	
		pH.	R.T. (hrs.).	pH.	R.T. (hrs.).
<i>B. aroideae</i>	Cortex	5.2	1.5	6.0	3.5
	Stele	6.4	1.1	7.2	1.5
<i>B. carotovorum</i> (i)	Cortex	4.9	> 24	4.6	> 24
	Stele	5.2	6.0	5.2	24

On the basis of the foregoing results one can suggest that *B. carotovorum* (i), as compared with *B. aroideae* in respect of pathogenicity, is handicapped by its tendency to an acid type of metabolism. This has the twofold result of slowing down its rate of multiplication and of setting up a reaction unfavourable to the working of its pectinase enzyme, the optimum of which is near pH 8. There may be other factors as yet undiscovered, but an understanding of the greater pathogenicity of *B. aroideae* is forthcoming from the analysis so far undertaken.

So far as *B. carotovorum* (ii) has been examined, it appears to offer no difficulties as it shows the same acid-forming tendencies as *B. carotovorum* (i) and in comparable conditions always gives weaker enzyme preparations than does the latter. Its lower grade of pathogenicity is therefore what would be expected.

The comparison between *B. aroideae* and *P. carotae*, however, offers difficulties. These are respectively the most and the least actively pathogenic of the four organisms studied, and this is true for all the host tissues tested. Nevertheless, as has already been mentioned (in connexion with Fig. 3), it has often happened that enzymic preparations of *P. carotae* come next to those of *B. aroideae* in activity. Furthermore, *P. carotae* tends to develop an alkaline reaction on suitable media, such as various plant decoctions, even more quickly than does *B. aroideae*. *P. carotae* reacts somewhat after the manner of *Botrytis cinerea* in that, for example, it very weakly attacks normal potato tissue, attacks injected tissues much more readily, and produces on various potato decoctions fairly strong enzymic preparations. It differs from *Bot. cinerea* in producing a pectinase enzyme which, so far as is known, is similar in its properties to the enzyme of the active parasite *B. aroideae*. For these reasons, therefore, a detailed comparison between *B. aroideae* and *P. carotae*, with a view to explaining the lack of pathogenicity of the latter, should give results of considerable interest. An analysis of this type is projected.

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A Technique for taking Successive Shadowgraphs of the Same Seedlings in Growth and Curvature Experiments with Oat Coleoptiles

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With Plates XVI and XVII and one Figure in the Text

ABSTRACT

A method is described for taking successive shadowgraphs of the same *Avena* seedlings, using an orange-sensitive paper and very low-intensity orange light such that whereas shadowgraphs can be made with one minute's exposure no phototropic or growth response can be detected as a result of one hour's unilateral exposure of intact seedlings. Phototropic curvatures produced by somewhat higher intensities of red light emphasize the importance of specifying not only the wave-length but also the intensity and duration of illumination used in experiments with *Avena* seedlings. When not subjected to the unilateral illumination the seedlings were grown under deep red light (wave-length $> 0.63 \mu$) at a total intensity of less than $\frac{1}{8}$ foot-candle; it must not be assumed that the technique would be satisfactory for seedlings grown in total darkness.

INTRODUCTION

THE great variability in growth rate and tropic curvature of the coleoptile shown by individual oat seedlings similarly treated is well known, although not infrequently it is ignored and mean values based on inadequate numbers of seedlings are used. It is therefore for many purposes desirable to be able to take successive measurements of growth or curvature on the same individuals, rather than on a fresh sample each time. Thus in experiments involving curvatures, in view of the difficulty of obtaining really straight seedlings to start with, doubtless partly due to nutation (Schneider and Went, 1938), it is most desirable to have initial records before treatment; it may also be advantageous to record the degree and kind of curvature at successive intervals after stimulation or application of growth substance. With the usual technique, in which white light is used for taking the shadowgraphs, this is unsafe since the exposure itself induces curvature. Schneider and Went (1938) therefore constructed their ingenious 'photokymograph', in which the decapitated coleoptiles are extended by the insertion of a pointer of wire on straw; successive shadowgraphs of a portion of each of these pointers are made by an intermittent flat beam of white light on photographic paper carried

on a revolving drum. This apparatus is, however, only suitable for *decapitated* coleoptiles and for curvature but not growth measurements; it is, moreover, somewhat elaborate for routine or class use. The essence of the new technique described below is simply to use a more or less panchromatic photographic paper for the successive shadowgraphs and to make the exposures with light passed through an orange or red filter such that no phototropic or growth response can be detected even when the photographically necessary exposure is multiplied many times.

METHOD

1. *Sensitive paper and colour filters*

A number of types of sensitive paper and colour filter were tried in preliminary experiments, but only those found most satisfactory need be mentioned, viz. Kodak reflex paper RP 15 and Dufay-Chromex cellulose acetate colour filters, Chromex 2436/C10 and 2202/C10. The filters appear unaffected by the high temperatures and high humidities to which they are subjected. The percentage transmissions of a single layer of each of these filters (data from Dufay-Chromex Ltd.) and the inferred percentage transmissions of a double layer of 2436/C10 are shown in Table I. Some similar data (from Handbook of Chemistry and Physics) for Schott and Genossen OG 2 glass filter 1 mm. thick, used by many continental workers, and for Corning 348 glass used, for example, by Schneider and Went (1938) for 'occasional illumination with an incandescent lamp', are tabulated for comparison. Some data kindly provided by Messrs. Kodak for the 'Wratten' series OA and No. 1 safelights are also included; these can only be very approximate owing to the inclusion of a light-diffusing material in the screens. The OA safelight is used in the routine method for coleoptile bending tests in this Institute and the No. 1 safelight was used in the present work (p. 255).

2. *Intensity distributions*

As far as phototropic and growth responses are concerned it is of course the intensity at various wave-lengths rather than the percentage transmission of the filter that should be known. For a physiological study of the responses of seedlings to different wave-lengths it would be desirable to express the intensity in absolute energy units at each wave-length, but the difficulties of such energy measurements are considerable and for defining the technique in a form such that it may easily be repeated by other workers luminosity units are preferable. These may be converted into energy units by anyone willing to undertake the necessary calculations (Wright, 1950, p. 40).

The method adopted was as follows: the total light intensity (E), with no filter, at the position in the shadowgraph box occupied by the seedlings, was measured in foot-candles with a photo-electric cell and meter calibrated for tungsten filament light at a colour temperature of 2,848° K; or in the case of the very low intensities used in the experiments with Chromex 2202/C10,

TABLE I
Percentage Transmission of Various Filters

Wave-length (μ).	Chromex			Schott & Gen.: OG 2 1 mm.*	Corning: 348.	Wratten Series:	
	2436/C10 Double.	Single.	2202/C10 Single.			OA.	No. 1.†
0.38	—	—	—	—	0.1	—	—
0.42	—	—	—	—	0.2	—	—
0.46	—	—	—	—	0.8	—	—
0.50	—	—	—	—	2.9	—	—
0.52	—	—	—	—	8.3	—	—
0.53	—	—	—	—	—	1.1	—
0.54	—	—	0.7	14	15.7	6.3	—
0.55	—	—	2.5	—	—	13.0	—
0.56	—	—	9.0	—	54.4	17.0	—
0.57	—	1.0	19.1	—	—	16.0	—
0.58	0.3	5.5	35.4	94	84.8	14.0	—
0.59	3.6	19.0	50.1	—	—	10.0	—
0.60	13.6	36.9	63.0	—	88.2	7.8	—
0.61	28.6	53.5	69.2	—	—	5.5	3.0
0.62	42.8	65.4	77.4	—	89.0	3.6	10.0
0.63	47.9	69.2	81.3	—	—	2.5	13.0
0.64	64.0	80.0	83.9	99	89.3	1.7	12.0
0.65	68.6	82.8	85.1	—	—	1.3	11.0
0.66	71.4	84.5	87.1	—	89.4	1.1	10.0
0.67	71.7	84.7	88.0	—	—	1.0	9.6
0.68	71.4	84.5	88.7	—	89.4	—	9.0
0.69	71.4	84.5	89.1	—	—	—	8.9
0.70	71.2	84.4	89.5	99	89.2	—	8.7
0.71	70.6	84.0	89.7	—	—	—	—
0.72	69.7	83.5	89.9	—	88.8	—	—

* Corrected for reflection losses, which might amount to 8% of the values tabulated.

† Approximate values, when viewed between 30 cm. and 120 cm. distance.

with a Holophane lumeter. The ratio (a_r/a) in which this total intensity was reduced by the filter was then estimated. For this purpose it was assumed that the colour temperature of the lamp filament would be $2,800^\circ$ K. Relative values for energy emission ($\mathcal{F}$) at this temperature by a perfect black body radiator were obtained from Int. Crit. Tables V, p. 240, for wave-lengths in steps of 0.01μ throughout the visible spectrum. These were converted into relative luminous flux by multiplication by the appropriate values of the C.I.E. standard photopic luminosity factor (Wright, 1950, p. 123) which gives the relative sensitivity of the eye of a 'standard observer' at each wave-length. The area (a) under the curve of luminous flux against wave-length so obtained was taken as proportional to E , the total intensity. The values of luminous flux at each wave-length were then multiplied by the appropriate relative transmission data for the filter and another curve plotted; the area (a_r) under this second curve being proportional to the total luminous flux with the filter in position, this last could now be estimated as $E(a_r/a)$. This total was then divided into ranges of wave-length 0.01μ wide. The data are presented in Table II both for a single and for a double layer of Chromex

2436/C10, and also for a single layer of Chromex 2202/C10 as used in the shadowgraph box (p. 264).

For comparison similar data are included for a Kodak safelight with 25-watt vacuum bulb at its rated voltage and Wratten series OA and No. 1 safelight screens. These data are for a distance of 30 cm. from the screen and also in the case of the OA screen with the area reduced to 2.5×18 cm. (see experiment of 15/2/51). The data are approximate because of the inexact percentage transmission data (p. 252). The total light intensity (E) without the filter was determined with the Holophane Lumeter.

TABLE II
Intensity in Foot-candles $\times 10^{-2}$ of Different Wave-length Ranges

λ (μ).	At position of seedlings in shadowgraph box, with Chromex filter:			30 cm. from Wratten safelight:			75 cm. from red lamp in dark- room.
	2436/C10		2202/C10*	OA (Area 23×18 cm. ²).	OA (Area 2.5×18 cm. ²).	No. 1 (Area 23×18 cm. ²).	
	Double.	Single.	Single.				
0.52—	—	—	—	0.2	0.02	—	—
0.53—	—	—	0.00	1.4	0.11	—	—
0.54—	—	—	0.03	4.9	0.39	—	—
0.55—	—	—	0.12	9.4	0.74	—	—
0.56—	—	0.1	0.32	10.7	0.84	—	—
0.57—	0.0	1.1	0.64	10.0	0.78	—	—
0.58—	0.5	3.8	0.97	7.8	0.61	—	—
0.59—	2.6	9.2	1.15	5.3	0.42	—	—
0.60—	6.0	13.5	1.17	3.7	0.29	0.3	—
0.61—	8.7	14.4	1.05	2.1	0.17	1.9	—
0.62—	8.3	13.2	0.80	1.1	0.09	4.1	—
0.63—	7.2	9.3	0.65	0.6	0.04	3.3	1.0
0.64—	6.1	7.6	0.46	0.3	0.02	2.0	3.5
0.65—	3.9	4.6	0.29	0.1	0.01	1.2	4.1
0.66—	2.3	2.8	0.17	0.0	0.00	0.6	3.6
0.67—	1.3	1.5	0.09	—	—	0.3	2.5
0.68—	0.6	0.8	0.05	—	—	0.1	1.5
0.69—	0.2	0.4	0.02	—	—	0.1	0.7
0.70—	0.1	0.2	0.01	—	—	—	0.4
0.71—	0.1	0.1	0.00	—	—	—	0.2
Total	47.9	82.6	8.08	57.6	4.53	13.9	17.5

* With 7 mm. diam. diaphragm in front of lamp.

3. *Temperature and humidity control*

The constant temperature room used was maintained at 25° C. and the relative humidity at about 85 per cent. Since difficulty is sometimes experienced with humidity control, the method used may be of interest. Water vapour was supplied by a small tank of water fitted with a constant level device and heated by a 400-watt element attached to the bottom. The heater element was actuated through a relay by a thermostat which was suitably placed in the room at some distance from the tank. A current of air was

directed over the water surface by a small A.C. desk fan in a wind tunnel. With the arrangement so far described the atmosphere was maintained saturated with water vapour, since there was necessarily a marked temperature gradient from the water tank to the thermostat and thus air left the water surface approximately saturated at a temperature above that of the rest of the room. This principle, of supplying heat through the water and having the thermostat in the air, has proved useful in experiments on storing onions in saturated atmospheres. In the present case, however, saturation was not desirable and a heating element, wired in parallel with the water heater, was therefore arranged as a grid between the fan and the water surface. This was chosen to give a near approximation to 85 per cent. R.H., and by adjusting the relative amounts of heat supplied by the 'wet' and 'dry' heaters almost any desired humidity should be obtainable. It should be mentioned that the humidity must vary slightly with the temperature outside the constant temperature room, a low ambient temperature resulting in the water in the tank having a higher average temperature and a high ambient temperature the converse effect. In practice this effect seems negligible for the present purposes, however.

4. Production of seedlings and allotting to treatments

Seedlings of 'Victory' oats were grown on agar slopes by the method of Weintraub (1938). The husked seeds were at first planted dry on the agar, as recommended, but it was later found advantageous to soak them first for 24 hours and to cover the test-tubes with inverted wider tubes to keep up the humidity during germination; the seedlings were grown thus before use for a total of about 66 hours, or in some experiments when germination was slow, about 90 hours. They were exposed to red light throughout, in this case supplied by a red glass Osram 60-watt gas-filled lamp rated at 240 V. and run on 200 V. This lamp was placed some 75 cm. vertically above the seedlings and was backed by a flat white reflector; the light was entirely confined to the deep red part of the spectrum (longer than 0.630μ) and the total intensity was 0.18 f.c. (Table II). In one experiment (15/12/49) the cover tubes were lined to within 5 mm. of the top with black paper to eliminate reflections which might interfere with the straight growth of the seedlings if the red light had any phototropic effect. No improvement in straightness was noticeable, however. A 'Wratten' safelight lamp with 25-watt vacuum bulb and 'Wratten' Series No. 1 red safelight screen (at least 45 cm. distant) was used occasionally for manipulation (see Table II).

The tubes containing the seedlings were stood vertically in racks of ten, double the number finally required being planted. After 66 hours the covers were removed and a sufficient number of the straightest seedlings of suitable height were selected. These were either allotted entirely at random, or else in descending order of size, to each of the treatments taken in random order; in the latter case in the analysis of the data the effects of the size groups could be eliminated both from the treatment means and the estimate of 'error'. This

procedure was found not to be worth while, however, as there was in general virtually no correlation between initial height (within the range investigated) and growth rate.¹

5. *Shadowgraphs and unilateral stimulation*

A strip of paper was stuck longitudinally on that side of the tube (the 'front') from which the coleoptile appeared most nearly straight. In general, shadowgraphs were made with the 'fronts' of the tubes facing the light and stimulation by light was applied with the tubes rotated through 90° in a noted direction.

The shadowgraphs and also stimulation by unilateral exposure to light were made by standing a rack of 10 tubes at one end of a black-lined box; the seedlings were illuminated from the other end through a horizontal slit, about 9×1 cm., by a 60-watt gas-filled 'single-coil' lamp, on its rated voltage, contained in a black-lined tin canister. The filament was about 65 cm. from the seedlings and the slit, which was covered with colour filters as required, about 50 cm. In the experiments using Chromex 2202/C10 filter (16 and 22/2/51) a diaphragm 7 mm. in diameter was placed immediately in front of the lamp, to reduce the light intensity and also to improve definition. Sensitive paper was held between two glass plates in spring clips about $1\frac{1}{2}$ cm. behind the seedlings.

6. *Procedure of a typical experiment*

A typical experiment will be described in detail to illustrate the method; for other experiments the results only will be presented in the next section and minor variations in technique, such as do not affect the conclusions, will not be discussed.

Experiment of 19/1/51. Technique. On 19/1/51 twenty seedlings were allotted to four sets (A_1 , B_1 , B_2 , and A_2) of five each as follows: the four longest seedlings were allotted at random, one to each of the four sets; then the four next longest, and so on. Sets A_1 and B_1 were placed in one rack; B_2 and A_2 in another. Thus in statistical analysis of the data both the average effects of size groups and of 'racks' could be eliminated both from treatment effect and estimate of error (see p. 257 and Table IV). The first shadowgraph, of A_1 , B_1 , was taken $90\frac{1}{2}$ hours from first imbibition of the seed. A double layer of Chromex 2436/C10 filter was used in the box and all shadowgraphs were given 1 minute's exposure.

A time schedule of the experiment is presented in Table III.

The treatment comparison was between the 'control' sets A_1 A_2 , which were given the minimum exposures necessary for adjustments in the box and taking the shadowgraphs, and the 'exposed' sets B_1 B_2 which were exposed in the box for an additional 60 minutes to the red light in a direction at right-angles to that from which the shadowgraphs were taken. During this 60

¹ The only experiment showing any such correlation was that of 15/2/51 (see pp. 258 and 263).

minutes the plants in the 'control' groups A_1 or A_2 were covered by black paper cylinders open at the top. When not in the box the racks were stood beneath the red lamp as during germination.

TABLE III
Experiment of 19/1/51. Times of Operations

Shadowgraphs and exposure to red light in box. Chromex 2436 (double).	Rack 1		Rack 2	
	A_1	B_1	B_2	A_2
No. 1 shadowgraph (1 min.)	10.25	10.25	11.36	11.36
Exposure (A_1 and A_2 covered; B_1 and B_2 turned 90° clockwise) 60 min.	—	10.28	11.40	—
No. 2 shadowgraph (1 min.)		11.28	12.40	
No. 3	11.30	11.30	12.42	12.42
No. 4	12.51	12.51	2.3	2.3
	2.12	2.12	3.24	3.24

The four shadowgraphs of Rack 1 are reproduced in Pl. XVI, Figs. 5, 6, 7, and 8.

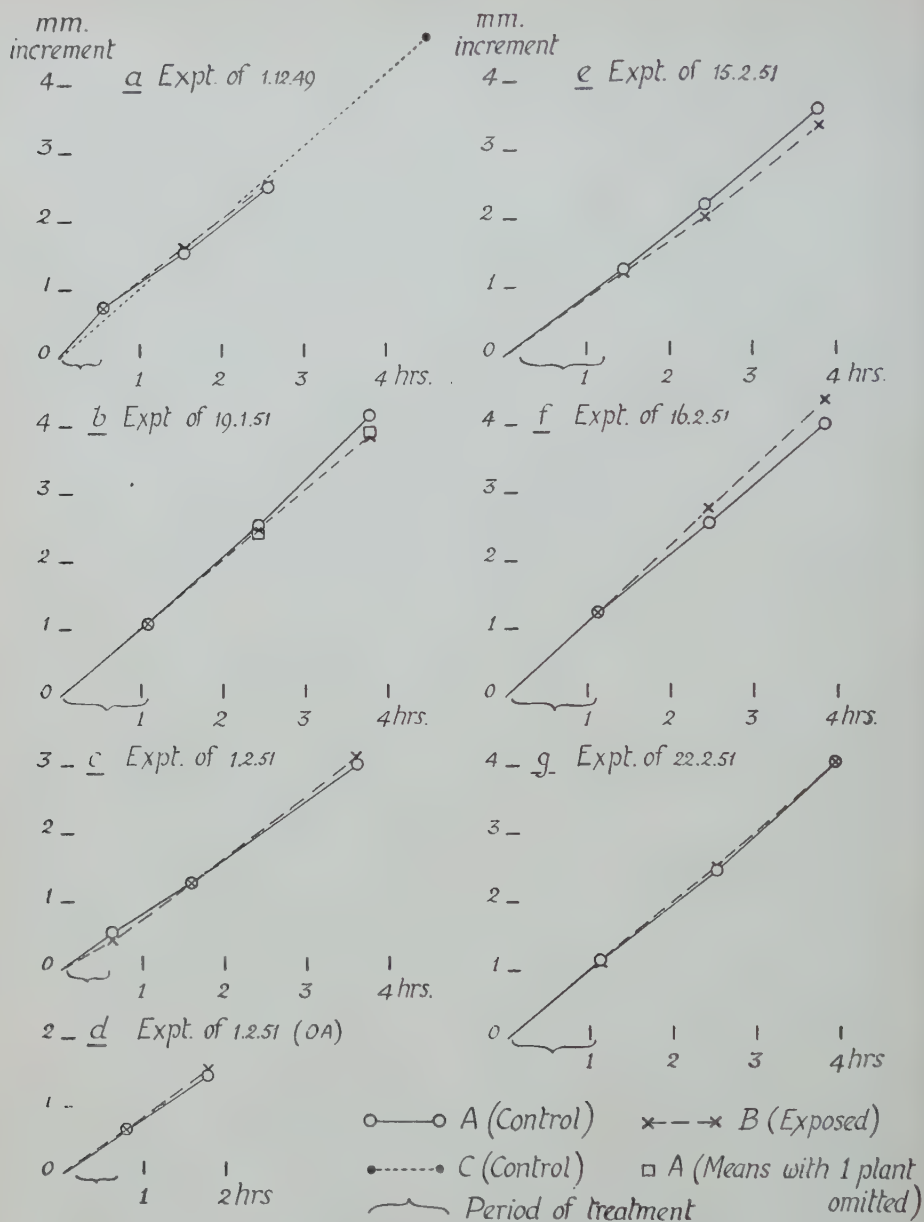
A different technique for the unilateral exposure was used in the experiments with the Wratten OA safelight (see pp. 262-3 below).

7. Growth measurement

No attempt was made to *measure* the curvatures which were shown in some of the shadowgraphs. Increments in length were measured on reflex prints in order to avoid damage to the originals. The method used consisted in marking with a pair of fine pointed dividers an arbitrary length above the tube top on the image of a coleoptile in the initial shadowgraph and also on each subsequent image. The heights above the mark were then measured, either with a dissecting microscope and calibrated micrometer eyepiece, or with an eyepiece micrometer scale, divided to 0.1 mm., laid flat on the print and examined with a 12× hand lens. A lower magnification was used with the dividers. In this way measurements repeatable to ± 0.1 mm. could be made. Where curvature had occurred the dividers were used on each side of the coleoptile and the mean increment was taken.

8. Statistical analysis of growth data

Inspection of the growth curves (Text-fig. 1, *a-g*) indicated that in most cases the growth rate of the 'treated' plants during the third period (shadowgraphs Nos. 3-4) tended to be very similar to those for the second period (Nos. 2-3). Analysis of variance including separation of linear regression and deviation from regression effects was therefore carried out, on increments above the initial heights, for the first two periods only, i.e. the period including the 'treatment' and that immediately following. This avoided 'dilution' of the 'deviation from regression' effects by the relative constancy of the growth rate over the second and third periods and hence rendered the detection of a treatment effect upon growth rate more likely, on the assumption that any such treatment effect would operate in opposite senses during and



TEXT-FIG. 1. Growth increments of exposed and 'control' plants.

immediately after the treatment. (If in fact the effect operated in the same sense during and after treatment it would be shown by the linear regression terms.)

The type of analysis carried out is indicated by the table for the experiment of 15/2/51 (Table IV). This was the only experiment in which a correlation

was shown between initial height and growth rate. It should be noted also that here and there were only four plants per set instead of the usual five, and thus three degrees of freedom for size groups instead of four.

TABLE IV

Experiment of 15/2/51. Analysis on Increment above Initial Height (in eye-piece scale divisions) for First Three Occasions

Variation.				Degrees of freedom.	Mean square.
Plants	Treatment (T)	.	.	1	0.1200
	Racks (R)	.	.	1	0.2134
	$R \times T$	.	.	1	0.0675
	(Size groups (S)	.	.	3	0.5481
	$R \times S$	.	.	3	0.0539
	$T \times S$	.	.	3	0.1517
	$R \times T \times S$	.	.	3	0.1325
				15	
	Occasion, linear (O')	.	.	1	64.0481
	$T \times O'$	.	.	1	0.1151
Plants $\times O'$	$R \times O'$	.	.	1	0.1266
	$R \times T \times O'$	.	.	1	0.0332
	$S \times O'$	.	.	3	0.2925
	$S \times T \times O'$	.	.	3	0.0968
	$S \times R \times O'$	.	.	3	0.0184
	$S \times R \times T \times O'$	.	.	3	0.0780
Plants $\times O''$	Occasion, deviations (O'')	.	.	1	0.0037
	$T \times O''$	.	.	1	0.0248
	$R \times O''$	.	.	1	0.0000
	$R \times T \times O''$	.	.	1	0.0019
	$S \times O''$	.	.	3	0.0152
	$S \times T \times O''$	.	.	3	0.0083
	$S \times R \times O''$	.	.	3	0.0145
	$S \times R \times T \times O''$	.	.	3	0.0053
				32	
				Total 47	

NOTES: In the first part of the table (15 degrees of freedom) the significance of average increment may be tested. Here size effect is significantly greater than the $S \times R$ interaction ($P < 0.05$), but no other effects reach significance.

In the second part of the table (32 degrees of freedom) the only significant effects are the average linear effect (O') and the linear term for size ($S \times O'$); the latter passes the $P 0.05$ level when tested against $S \times R \times O'$, which suggests a real difference in mean growth rate for the different size groups, but is not significant against $S \times R \times T \times O'$ or the two variances combined. The linear term for treatment ($T \times O'$) does not approach significance, nor does the $T \times O''$ variance or any of the other deviation effects. There is thus no good evidence of treatment differences either in mean growth rate ($T \times O'$) or in change of growth rate between the first and second periods ($T \times O''$).

The bracketed terms indicate the type of analysis used where no separation into size groups was made.

In fitting the regressions, the mean duration of each period (from No. 1 to No. 2 and from No. 2 to No. 3) was used. Actually the periods differed

slightly for the two racks in some experiments. This may have given rise to spurious 'rack' effects, but the linear and deviation terms for treatment ($T \times O'$ and $T \times O''$) which are of primary interest will not have been affected nor will their appropriate errors, viz. $\text{Plants} \times O'$ and $\text{Plants} \times O''$ respectively where the plants had not been separated into size groups; or where such separation had been made, $S \times R \times T \times O'$ and $S \times R \times T \times O''$.

RESULTS

Experiment of 1/12/49

A single layer of Chromex 2436/C10 was used in the box, both for the shadowgraphs (5 seconds exposure) and for stimulation. There were three 'treatments': the 'control' sets $A_1 A_2$, the 'exposed' sets $B_1 B_2$ which were stimulated for 30 minutes, and a second pair of 'control' sets $C_3 C_3$ which were shadowgraphed only at the beginning and end of the experiment in order that the growth rate might be measured after only an initial few seconds' exposure to the light in the box. These last were not, however, strictly comparable with A and B as they were in a different rack which was not in the box at the same time.

Shadowgraphs of $B_2 A_2$ taken before the treatment, immediately after it, and then after a further one and two hours are reproduced in Pl. XVI, Figs. 1, 2, 3, and 4. They show that immediately after the 30 minutes' exposure (Fig. 2) there was no noticeable bending of the coleoptiles, but an hour later (Fig. 3) marked curvature of the exposed (B) coleoptiles had occurred. After a further hour (Fig. 4) the bend had travelled down the coleoptiles and in the last shadowgraphs taken 2 hours later (not reproduced) it was below the test-tube tops. Similar results for curvature are to be seen in the $A_1 B_1$ shadowgraphs (not reproduced).

The mean increments in length for the three treatments are shown in Text-fig. 1a. Analysis of variance (for the A and B treatments only) shows a complete absence of significant treatment effects, either in the linear or deviation terms. The average deviation from regression is, however, very significant ($P < 0.01$) showing a real fall in growth rate after the period in the box; the reason for this is not apparent.

Experiment of 15/12/49

Two layers of Chromex 2436/C10 were used in the box, all shadowgraphs having 1 minute's exposure. There were three treatments, viz. 'control' sets $A_1 A_2$, 'exposed' sets $B_1 B_2$ stimulated for 10 minutes, and another pair of 'exposed' sets $D_3 D_3$ stimulated for 50 minutes. This third pair of sets were in a different rack by themselves and again therefore were not strictly comparable with A and B .

Shadowgraphs were taken before the treatments, but not again until about 80 minutes after the completion of treatment. Further shadowgraphs were taken after another 3 or 4 hours. The A and B sets gave no indication of curvature, but in the two D sets which had been stimulated for 50 minutes

four or five of the seedlings gave a slight suggestion of curvature in the expected direction.

The mean growth rates for the sets of five seedlings are presented in Table V. Statistical analysis of the data for the first period, which included the unilateral exposure to red light in the box for treatments *B* and *D*, shows a significantly higher growth rate for treatment *D* than *A*. Owing to the absence of a shadowgraph taken soon after the end of the exposure period there is doubt as to whether the faster growth occurred during or after the treatment or both; this omission and the non-orthogonal design of the experiment make it impossible to apply the type of analysis described above (p. 258) or even to eliminate rack effects in the analysis.

TABLE V

Experiment of 15/12/49. Mean Growth Rates in mm./hour (Chromex 2436/C10-double)

	Period.	<i>A</i> (control).	<i>B</i> (10 minutes' exposure).	<i>D</i> (50 minutes' exposure).
1st period (incl. exposure)	Rack 1 (103 min.)	0.90	1.25	—
	Rack 2 (93 min.)	1.11	1.04	—
	Mean	1.01	1.15	—
	Rack 3 (134 min.)	—	—	1.27
	Rack 3 (134 min.)	—	—	1.32
	Mean	—	—	1.30
<i>D</i> significantly $> A$ $P < 0.01$				
2nd period	Rack 1 (256 min.)	1.00	0.98	—
	Rack 2 (252 min.)	1.14	1.11	—
	Mean	1.07	1.05	—
	Rack 3 (198 min.)	—	—	1.00
	Rack 3 (198 min.)	—	—	0.98
	Mean	—	—	0.99

A further experiment orthogonally designed and with shadowgraphs taken immediately after a long exposure to the unilateral red light seemed desirable. Such an experiment is described below.

Experiment of 19/1/51

The technique of this experiment has already been described in detail (p. 256). Shadowgraphs Nos. 1, 2, 3, and 4 for Rack 1 (see Table III) are reproduced in Pl. XVI, Figs. 5, 6, 7, and 8. No curvature was visible immediately after the 60 minutes' exposure (Fig. 6), but 81 minutes later (Fig. 7) slight but definite bending of the exposed (*B*) coleoptiles in the expected direction, left to right, had occurred. The next shadowgraph (Fig. 8) taken after a further 81 minutes suggested that the bends had travelled down the coleoptiles as in

Fig. 4. The shadowgraphs of Rack 2 showed similar effects, but the bending due to exposure was less marked than in Rack 1.

The mean increments for the two treatments are plotted in Text-fig. 1*b*. The apparent difference between the treatments was almost entirely due to one 'control' plant which grew abnormally rapidly, as is shown by the extra points plotted with this plant omitted from the means. It was thus clear that treatment had no significant effects on growth rate in this experiment and statistical analysis was deemed unnecessary.

In view of the definite bending response to a sufficient dosage of light filtered through the very red double layer of Chromex 2436/C10, it was thought desirable to attempt to define rather more closely the maximum 'safe' exposure, which apparently lay between 10 minutes and 50 minutes.

Experiment of 1/2/51

The technique resembled that of the previous experiment except that the *B* seedlings were exposed for 30 minutes instead of 1 hour; a double layer of Chromex 2436/C10 was used. The first three shadowgraphs of Rack 2, taken before treatment, immediately afterwards and 1 hour later, are reproduced in Pl. XVII, Figs. 9, 10, and 11. No definite bending is seen, with the exception of the *B* plant on the extreme left of Fig. 10; this might, however, have been the result of nutation. In the shadowgraphs of Rack 1 (not reproduced) two of the *B* plants show a similar bend developed 1 hour after the end of exposure, but so do two of the *A* plants which were not exposed. The bending is of little significance therefore.

Analysis of the growth data showed no significant linear effects except the average slope; the deviation terms for treatment and treatment $\times$ racks interaction were, however, significant ($P < 0.05$). The mean increments plotted in Text-fig. 1*c* show that the curve for the treated plants (*B*) has a marked increase of slope for the second period as compared with the first; thus the light passed through the double 2436 filter apparently decreased growth during the unilateral exposure but resulted in an increase afterwards. For the control plants (*A*) there is a slight bending of the curve in the opposite direction and the possibility of a period of darkness affecting the growth of these must be borne in mind. The significant interaction takes the form that these effects were much larger in Rack 1 than in Rack 2.

It is most remarkable that a significant treatment effect upon straight growth should be found in the absence of any apparent effect on curvature, especially as the converse occurred in the experiment of 19/1/51 with double the time of exposure to the same light source. The results of the present experiment should therefore be accepted with caution unless confirmed by further experimentation.

Experiment of 1/2/51 with OA safelight

In this experiment the shadowgraphs were taken in the box with Chromex 2436/C10 filter (two layers) as in the experiment of the same date just

described, but the unilateral stimulation was given by standing the two racks at 30 cm. distance from the Wratten Series OA screen of a Kodak safelight with 25-watt vacuum bulb on its rated voltage. The $A_1 A_2$ (control) seedlings were covered with black paper cylinders while the $B_1 B_2$ seedlings were exposed for 30 minutes. Shadowgraphs were taken immediately before and after the treatment period and then an hour later. There was no evidence of phototropic curvature, and the mean increments (Text-fig. 1*d*) show no effect of treatment on straight growth; this in spite of the relatively high intensity in the green-yellow (0.54–0.57 μ) where the double layer of Chromex 2436 transmitted virtually nothing (Table II).

Experiment of 15/2/51 with OA safelight

It was thought that the lack of phototropic curvature in the previous experiment might have been due to the very wide angle subtended at each plant by the 23-cm. wide safelight screen. In the present experiment, therefore, a series of parallel vertical black cardboard partitions was arranged so that the light from the OA safelight reached each exposed (*B*) seedling between a pair of partitions 2.5 cm. apart; thus the area to which the seedling was exposed, at 30 cm. distance, was 2.5 cm. wide $\times$ 18 cm. high. This also had the effect of reducing the intensity in the ratio of 1:12.7 (Table II). It was found that at this reduced total intensity a satisfactory shadowgraph could be made with 5 to 10 seconds' exposure. The $B_1 B_2$ seedlings were given 1 hour's stimulation in this way, the $A_1 A_2$ seedlings being covered as usual. Owing to the limited width of the safelight screen, only four seedlings could be included in each set. Shadowgraphs were taken before and after the treatment, and then after further 1- and 1½-hour intervals. Chromex 2202/C10 was used in the box for the shadowgraphs with a 7-mm. diaphragm in front of the lamp (see experiment of 16/2/51, p. 264). The first three shadowgraphs of Rack 2 are reproduced in Pl. XVII, Figs. 12, 13, and 14. There is no evidence of phototropic response either here or in the shadowgraphs of Rack 1 (not reproduced). In this experiment the expected direction of curvature is from right to left. Note that the third and fourth B_2 seedlings were interchanged in position between the taking of the first and second shadowgraphs.

The mean increments are shown in Text-fig. 1*e*. The statistical analysis has already been presented in detail (Table IV) and shows that treatment was without significant effect.

The lack of bending or growth response to the very yellow light of the OA safelight (at an intensity sufficient to give a satisfactory shadowgraph on RP 15 paper with only 10 seconds' exposure) suggested that instead of using a relatively intense light and two layers of Chromex 2436/C10 in the box for shadowgraphs it might be safer to use a more actinic orange light at a much lower intensity. By 'safer' is meant giving a higher ratio of the maximum 'safe' exposure to the minimum exposure needed to make a shadowgraph. The following experiments were carried out to test this.

Experiment of 16/2/51

In this experiment a single layer of the orange filter Chromex 2202/C10 was used in the box and a diaphragm 7 mm. in diameter reduced the total intensity to 0.08 f.c. (see Table II); this diaphragm was selected as the smallest that would allow of shadowgraphs being made with one minute's exposure. The *B* seedlings were exposed in the box for 1 hour. The shadowgraphs, taken before, immediately after treatment and then at 80-minute intervals, gave no evidence of a phototropic response, such bending as was seen occurring in both the *A* and *B* sets and in either direction. The mean increments are shown in Text-fig. 1f. Statistical analysis showed no significance of treatment, or indeed of any effects except the average slope.

Experiment of 22/2/51

This was a repetition of the previous experiment, with similar technique, except that the ten longer seedlings were allotted (at random) to the two treatments in Rack 1 and the ten shorter in Rack 2. The first three shadowgraphs of Rack 1 are reproduced in Pl. XVII, Figs. 15, 16, and 17. There is no evidence of phototropic response either in these or the shadowgraphs of Rack 2 (not reproduced).

The mean increments are presented in Text-fig. 1g: again there are no significant effects except the average slope.

DISCUSSION

It may be doubted whether any wave-length in the visible spectrum is entirely inactive in affecting the growth and phototropic curvature of the *Avena* seedling (cf. Galston, 1950). The small but definite phototropic response to an hour's exposure using a double layer of the red Chromex 2436/C10 filter (which cuts off virtually all light of wave-length less than 0.580μ) with a total intensity of less than half a foot-candle, indicates the importance of specifying not merely the colour but also the intensity and duration of the illumination in coleoptile experiments. An hour's exposure using a single layer of the much more orange Chromex 2202/C10 filter, with a total intensity reduced to 0.08 f.c., gave no detectable curvature or growth response, but the actinic value of the illumination was sufficient for the taking of shadowgraphs with 1 minute's exposure. Such a reduction in luminosity represents an even greater reduction in energy units owing to the greater sensitivity of the eye to yellow than to red light, and since the photographic material used is *very* much more sensitive to yellow than red it would seem preferable to use low-intensity orange light both for visual and photographic purposes. As far as visual purposes are concerned this has already become standard practice in the use of such filters as OG 2, but the intensity is seldom specified. It seems likely that a further reduction in total energy could be achieved, while retaining adequate illumination both for visual and photographic purposes, by using a filter with a relatively narrow band in the yellow-

green to orange such as Wratten Series *OA*. This is suggested by the apparent lack of response to light from the *OA* safelight with a total intensity of about 0.05 f.c. and this intensity could certainly have been much reduced both for visual work and shadowgraphs. The ideal might well be very low intensity monochromatic light at the point of maximum sensitivity of the eye, viz. about 0.555μ . This is almost the point of maximum transmission of the *OA* filter.

The outcome of these investigations is then to suggest the use of a narrow band filter at about 0.560μ and the lowest intensities that will give satisfactory vision in the coleoptile chamber and a short enough exposure with RP 15 paper in the shadowgraph box. For taking shadowgraphs, however, a single layer of Chromex 2202/C10 filter with a total light intensity of 0.08 f.c. may be considered satisfactory, since 60 times the exposure needed for one shadowgraph causes no significant curvature or growth response. It should therefore be safe to take a long series of shadowgraphs of the same seedlings without risk of affecting their growth or curvature appreciably.

The results with a double layer of Chromex 2436/C10 filter are less clear cut, for 1 hour's exposure caused definite curvature but no appreciable growth response (experiment of 19/1/51), whereas half an hour's exposure caused a significant growth response in the absence of any consistent curvature (experiment of 1/2/51). The latter result, if substantiated, would be of considerable interest, but further experimentation would be necessary before it could be accepted without reserve and the aim of the present investigation was to develop a satisfactory technique—this has been achieved by the use of Chromex 2202/C10.

It should be emphasized that all these results are for seedlings growing in deep red light; no tests have been made with seedlings grown in darkness. Any growth responses found in the present experiments therefore represent a *differential* effect as between the deep red vertical illumination in the dark room on the one hand and the horizontal illumination used in the shadowgraph box for the 'treated' plants, or the much reduced vertical illumination for the 'controls', on the other. Such a differential response seems much less likely to occur than a direct effect of illumination upon the growth of seedlings previously grown in complete darkness and it must not be assumed that the technique developed would be safe for the latter.

It should also be emphasized that all the experiments were carried out with intact seedlings; any technique of taking shadowgraphs found safe for these would presumably be safe for decapitated seedlings which are known to be much less sensitive at least as far as phototropic response is concerned.

The very small deviations from linearity shown in most of the 1951 experiments (e.g. those with Chromex 2202/C10 filter) not only indicate in the case of the treated plants that here any effect of treatment on growth rate was extremely small, but also that even with so few as ten plants per treatment reliable results can be obtained when the same individuals are used throughout. It is worthy of note that the mean growth rate approximated closely to the value of 0.9

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mm./hr. given by Weintraub (1938) for the same temperature. The linear regression coefficients (for the first two periods) are shown in Table VI.

TABLE VI

Mean Linear Regression Coefficients (mm./hr.) of Height on Time for the First Two Periods of Each Experiment (A and B Treatments)

Experiment.	Regression coefficient.
1/12/49 (2436 single)	1.00
15/12/49 (2436 double)	1.07*
19/1/51 (")	1.00
1/2/51 (")	0.78
1/2/51 (OA)	0.81
15/2/51 (OA)	0.87
16/2/51 (2202)	1.09
22/2/51 (2202)	0.99
Mean	0.95

* For first period (98 min.) only.

The writer wishes to thank Dr. W. D. Wright, of the Physics Department, Imperial College, for advice on the photometric problems involved and for determining the transmission curve of the red lamp used in the dark-room.

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DESCRIPTION OF PLATES

Illustrating O. V. S. Heath's paper on 'A Technique for taking Successive Shadowgraphs of the Same Seedlings in Growth and Curvature Experiments with Oat Coleoptiles'.

PLATE XVI

FIG. 1. *Experiment of 1/12/49*. Initial shadowgraph of Rack 2 before treatment; B_2 on left, A_2 on right.

FIG. 2. The same 32 minutes later, after the five B_2 seedlings have been exposed for 30 minutes to unilateral illumination (Chromex 2436/C10 filter, single layer).

FIG. 3. The same, 1 hour later.

FIG. 4. The same, 1 hr. 2 min. later.

FIG. 5. *Experiment of 19/1/51*. Initial shadowgraph of Rack 1 before treatment; A_1 on left, B_1 on right.

FIG. 6. The same, 1 hr. 5 min. later, after the five B_1 seedlings have been exposed for 1 hour to unilateral illumination (Chromex 2436/C10 filter, double layer).

FIG. 7. The same, 1 hr. 21 min. later.

FIG. 8. The same, 1 hr. 21 min. later.

PLATE XVII

FIG. 9. *Experiment of 1/2/51*. Initial shadowgraph of Rack 2 before treatment; B_2 on left, A_2 on right.

FIG. 10. The same, 35 minutes later, after the five B_2 seedlings have been exposed for 30 minutes to unilateral illumination (Chromex 2436/C10 filter, double layer).

FIG. 11. The same, 58 minutes later.

FIG. 12. *Experiment of 15/2/51*. Initial shadowgraph of Rack 2 before treatment; B_2 on left, A_2 on right.

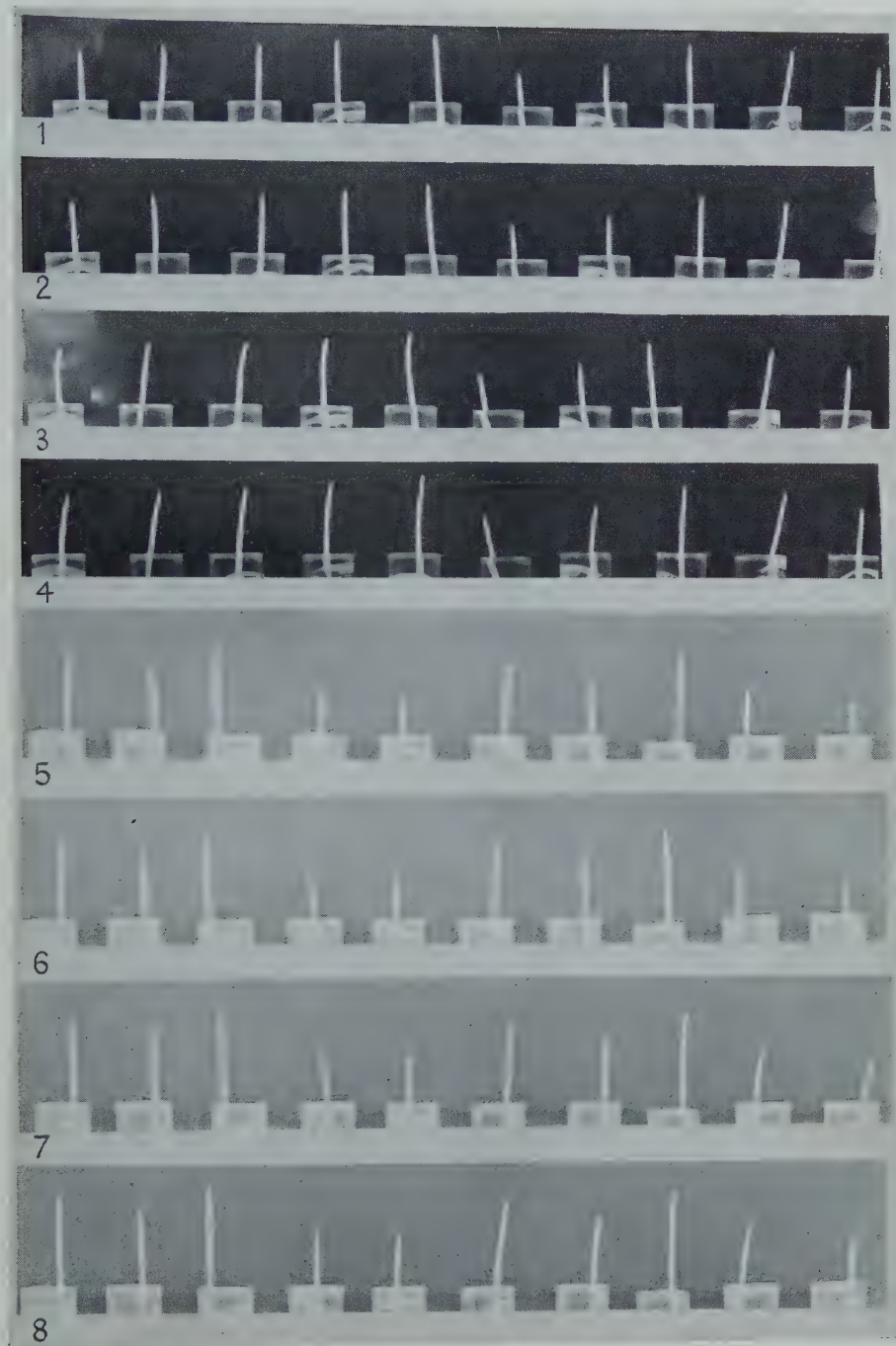
FIG. 13. The same, 1 hr. 25 min. later, 13 minutes after the completion of 1 hour's exposure of the four B_2 seedlings to unilateral illumination (OA safelight; area 2.5×18 cm.²). Note that the third and fourth B_2 seedlings have been interchanged since Fig. 12.

FIG. 14. The same, 1 hour later.

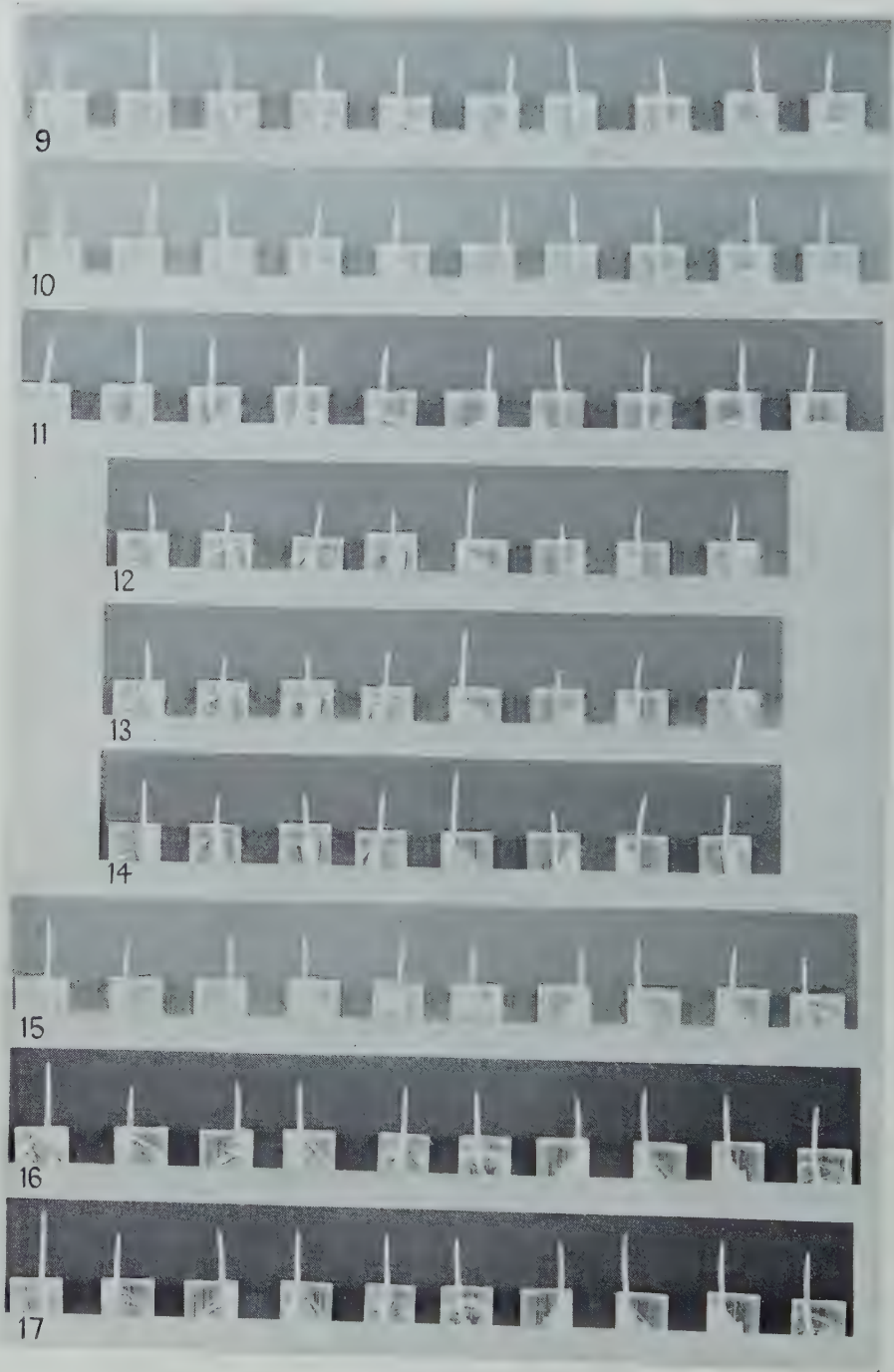
FIG. 15. *Experiment of 22/2/51*. Initial shadowgraph of Rack 1 before treatment; A_1 on left, B_1 on right.

FIG. 16. The same, 1 hr. 8 min. later, after the five B_1 seedlings have been exposed for 1 hour to unilateral illumination (Chromex 2202/C10, single layer).

FIG. 17. The same, 1 hr. 20 min. later.



O. V. S. HEATH



O. V. S. HEATH

Addenda Clavariaceae

I. Two new Pteruloid Genera and *Deflexula*

BY

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With fifteen Figures in the Text

ABSTRACT

The genera *Allantula* (one sp.) and *Parapterulicium* (two spp.) are described from Brazil. The first has decumbent, cystidiate and unbranched fruit-bodies. The second has gloeocystidia and dichophyses, neither of which have yet been recorded in Pteruloid genera.

In the revision of *Deflexula*, ten species are recognized, four being transferred from *Mucronella* and *Pterula*, and three being described as new, namely, *D. major*, *D. mangiformis*, and *D. sulcispora*.

SINCE the publication of my monograph I have had the opportunity of collecting, though sporadically, fungi in South America and of studying a further large number of herbarium specimens, both types and doubtful or unnamed specimens. In all I have examined another 550 collections, representing 21 out of the 26 Clavarioid genera that I have recognized. The only case where generic description may need modification concerns *Physalacria* and *Hormomitaria*, but these cover such curious fungi, requiring field-observation on the direction of growth of the fruit-body, that they are difficult to evaluate from dried material. That the other genera have withstood this test shows how they parcel out clavarioid fungi into homogeneous and exclusive groups, as a natural classification should, whereas the old system, based on European species, had not a single exclusive genus when applied to the world-flora: it was not only highly artificial, but thoroughly unscientific.

The criteria for the genera which I recognize are distinguishable without difficulty, provided, as I have always assumed, the mycologist uses a high enough magnification, such as the ordinary oil-immersion lens supplies: without such, little structural detail can be made out from dried material: with such, only the over-sublimated or the very old material may be refractory. I am unable to sympathize with the criticism that it is difficult to decide on the presence or absence of clamp-connexions: it shows that insufficient magnification has been used and that many other uncertainties must also have been unresolved.

In publishing my results I have begun with the Pteruloid fungi because I found them to be extremely abundant in South America and more varied

than I had observed in the eastern tropics. By contrast I did not meet with a single *Thelephora* (clavarioid or stereoid) in Brazil, though several of these, as will be mentioned in my monograph of the genus, are common in Malaya. It is unwise, however, to draw distinctions in mycological geography without prolonged residence over several fungus-seasons in tropical countries, because of the ease with which fungi can be overlooked. Thus, while I met also in Brazil many agarics and polypores with which I was familiar in the East, there seemed to be a notable paucity of certain genera, but I hesitate to draw conclusions because I did not for certainty collect in the height of any fungus-season in South America, unless it was in November and December 1948, on the mountain Corcovado in Rio de Janeiro.

I am greatly indebted to the following mycologists for the loan of material: Professor R. E. D. Baker (Trinidad, B.W.I.), Dr. R. W. G. Dennis (material from B.W.I.), Dr. M. Doty (Evanston, Illinois), Professor R. Heim (Paris), Dr. J. C. Lindquist (collections of Spegazzini, La Plata, Argentina), Dr. R. A. Maas-Geesteranus (Indonesian material, Leiden), Professor G. W. Martin (tropical and North American material, Iowa), Dr. A. Pilat (collections of Velenovsky, Prague), Dr. J. A. Stevenson (Lloyd Mycological Collections and material from U.S. Dept. Agriculture Mycological Collections, Beltsville, Maryland), Dr. G. Taylor (British Museum), and Miss E. M. Wakefield (Kew).

Duplicates of my own material have been supplied, so far as possible, in return, and my own collections are deposited in the herbarium of the Botany School, Cambridge.

ALLANTULA gen. nov.

(Gr. ἀλλᾶς, a sausage)

Receptacula parva, decumbentia, allantiformia, ut segmenta rhizomorpha evoluta, saepissime paulum curvata, ceraceo-carnosa: sporis albis, levibus, ellipsoideis, aguttatis: cystidiis plus minusve immersis, tenuiter tunicatis: hymenio incrassato: hyphis biformibus, ut in *Pterula*: ad lignum: Brazil: typus—*A. diffusa*.

Fruit-bodies dimitic, developed on slender rhizomorphs as small, sausage-like, decumbent, intercalary swellings, generally curved, rarely bifid or anastomosed, waxy-fleshy.

On dead branches; Brazil (1 species).

Spores white, smooth, aguttate.

Basidia 4-spored: hymenium thickening: cystidia thin-walled, becoming embedded.

Generative hyphae clamped: skeletal hyphae only in the rhizomorphs, colourless, the walls thickened, sometimes branched, unlimited, or merely as intercalary portions of the generative hyphae.

The very slender, dimitic rhizomorphs of this fungus grow over the under-surface of the dead wood or bark, developing many adventitious branches, mainly in acropetal order. They consist of longitudinal generative and skeletal

hyphae and are not corticate or sclerotoid, though some of the superficial generative hyphae give off small swellings, as though they were rudimentary caulocystidia. The hyphal tips at the growing-points of the rhizomorphs are $1.2-2.5 \mu$ wide.

The surface of the rhizomorph becomes fertile in places by the outgrowth

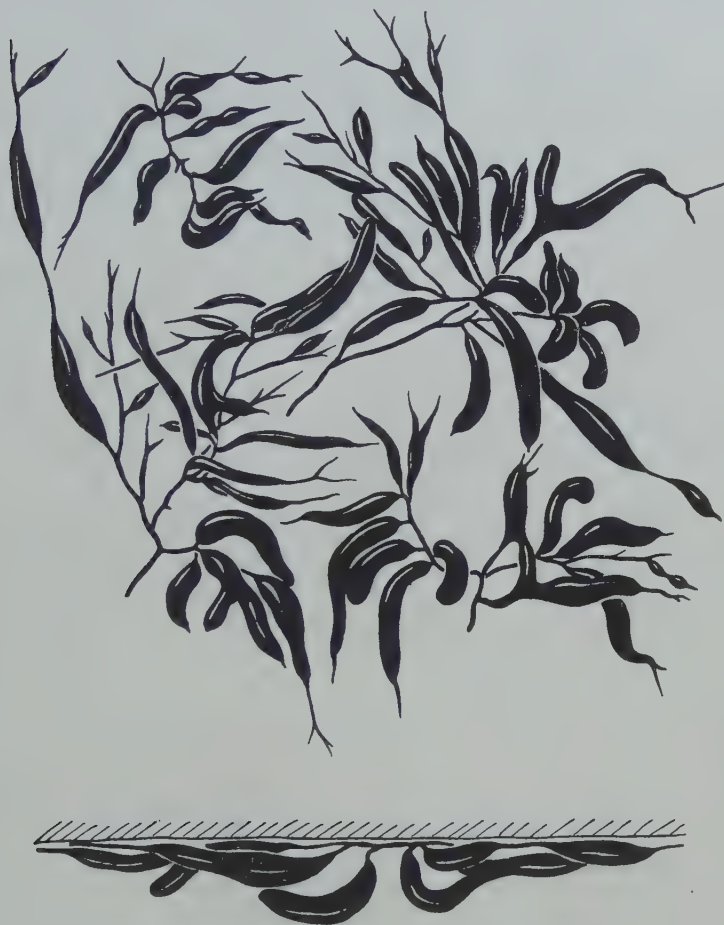


FIG. 1. *Allantula diffusa*. $\times 5$.

of laterals from the generative hyphae, some becoming basidia, others cystidia. Then, by the sympodial growth of the subhymenial hyphae, the hymenium thickens, being fertile from the first. The cystidia are of limited growth, becoming embedded as the hymenium thickens, but new cystidia are continually added. Sometimes the leader of a rhizomorph and its branch become fertile at the same time and coalesce to give the appearance of branched fruit-bodies. There is no adventitious branching of the fruit-body itself; neither is there fanning out of the rhizomorph, nor Corticioid expansion.

The fruit-bodies are thus intercalary, decumbent, and, apparently, ageotropic. I did not see any rhizomorphs on the upper sides of the sticks on which the fungus was growing. I collected it on one occasion only, and it seems to be rare, though easily overlooked.

The dimitic structure, spores, and cystidia show that the fungus belongs to the Pteruloid series, many species of which develop slender rhizomorphs. *Allantula* seems to be a degenerate state in which the form-factors of the Clavarioid fruit-body have disappeared, except for concrescence of the mycelial hyphae, adventitious branching, and thickening hymenium. If the rhizomorphs developed fan-like tissue, as a further degeneration from strict concrescence, a fan-like Corticioid fruit-body would result, with confluence of the hymenia: the possibility opens up other vistas of Pteruloid Corticioid derivatives than the direct resupinate forms of *Pterulicium* and *Parapterulicium*.

A. diffusa sp. nov.

Figs. 1, 2

Receptacula ad $5 \times 0.5-0.7$ mm., pallide alba, dein subochracea: rhizomorphis $25-70 \mu$ latis: sporis $6.5-8 \times 3.2-3.7 \mu$: cystidiis $30-120 \times 7-12 \mu$, subcylindricis vel subventricosis, haud incrustatis: hymenio ad 300μ incrassato: hyphis structilibus $2.5-5 \mu$ latis: hyphis tenuiter tunicatis septato-fibulatis: Corner 764 (Rio de Janeiro, Corcovado, pr. Paineiras).

Fruit-bodies reaching $5 \times 0.5-0.7$ mm., *pallid white, then pale drab watery ochraceous*, crowded or subcaespitose, often curved, rarely anastomosing or bifid, sometimes constricted, *waxy fleshy*, putrescent: rhizomorphs $25-70 \mu$ wide, white, then pallid ochraceous.

On the underside of a fallen branch in the forest: Brazil (Rio de Janeiro, Corcovado, pr. Paineiras, c. 500 m. alt., Nov. 28, 1948, Corner 764).

Spores $6.5-8 \times 3.2-3.7 \mu$, white, smooth, ellipsoid or subamygdaliform, thin-walled, aguttate, not amyloid.

Basidia $28-38 \times 6.5-7.7 \mu$, clavate: sterigmata 4, $3.5-4 \mu$ long.

Cystidia $30-120 \times 7-12 \mu$, subcylindric to subventricose (especially the first-formed cystidia), blunt or subcapitate, sometimes waisted, thin-walled, colourless, not encrusted, the apex usually with a cap of dense cytoplasm (especially in growing cystidia), projecting to 25μ , eventually immersed.

Hymenium thickening to 300μ : subhymenial hyphae $1.5-2.5 \mu$ wide at first, enlarging to 5μ wide in the thickened hymenium.

Generative hyphae $2-5 \mu$ wide, clamped, thin-walled, often branched from the clamp: skeletal hyphae $2.5-5 \mu$ wide, or expanded in places to 9μ wide, the walls to 1μ thick, sometimes branched and appearing bifurcate, either of unlimited growth or merely as intercalary portions of the generative hyphae, often kinked and of uneven width: crystals, to 20μ wide, as rather irregular lumps, abundant in the rhizomorphs.



FIG. 2. *Allantula diffusa*: hymenium (thickened) and hyphae. $\times 1,000$.

DEFLEXULA

To this characteristic tropical genus I have transferred *Mucronella argentina*, *Pterula pennata*, *P. subsimplex*, and *P. Vanderystii*, and I have added three new species, *D. major*, *D. mangiformis*, and *D. sulcispora*. Three of these additions have relatively large and branched fruit-bodies, and it is necessary to modify the concept of the genus, which becomes in essence *Pterula* with positively geotropic habit of the fruit-body. In *D. major* and *D. subsimplex* (Figs. 3, 5, 6, and 7) the fruit-bodies have multifid and bifid branching as well as adventitious. As dried specimens, they were mistaken for erect-growing species of *Pterula*, but attention to the position of the hymenium shows that the branches must have been decurved, as, indeed, the unilateral development of the fruit-bodies and their unilateral adventitious branching suggest. The hymenium, in their case, is on the inside of the curvature of the branches, which had therefore grown downwards, whereas in *Pterula* it is, when unilateral, on the outside of the curvature of branches which grow upwards. Sometimes erect fruit-bodies of *Pterula* grow from the underside of the substratum, but they soon acquire a more or less centric form which helps to distinguish them from the one-sided condition in *Deflexula*. Thus, the fruit-bodies of *D. major* and *D. subsimplex* differ from those of *Pterula* in having positively geotropic branch-tips, though the stem, or original axis, appears to be diageotropic. In *D. pennata* (Fig. 4) this diageotropic axis is strongly developed and the positively geotropic branches which arise along its underside are short and simple. Thus, *D. pennata* presents a transitional form between the erect, or negatively geotropic, condition of *Pterula* and that of *Deflexula*. If the growth of the axis of *D. pennata* were curtailed and its tip deflexed, the condition in *D. fascicularis* would be reached: alternatively, limitation of the branches of *D. major* or *D. subsimplex* would give the same effect. Further restriction to a short, spine-like axis proliferating adventitiously from the base would give the condition in the simple fasciculate species, as *D. sulcispora* (Fig. 9). Thus, as the erect-growing fruit-bodies of *Pterula* simplify to the unbranched filiform axis, as in *P. gracilis*, those of *Deflexula* reduce to the spuriously hydroid form which I recognized in my monograph. The 'spines' of this form, however, are branches of the fruit-body, those of *Hydnum* being appendages developed in a marginal field of growth (as the positives of the polyporoid tubes). *Deflexula* is, therefore, a characteristic offshoot of *Pterula* with its own end-product.

A second character of the genus is the large size of the spores (and basidia) compared with that of *Pterula*. *D. Vanderystii* seems an exception, having spores only $6 \times 5 \mu$, but globose spores are not known in any genuine *Pterula*, though occurring in two other species of *Deflexula*.

A third character is the slightly angular outline of the dried spore as seen in optical transverse section. I did not notice this in the living material of *D. fascicularis*, *D. lilaceo-brunnea*, and *D. sulcispora*, which I examined, but it is evident in the dried material and may, therefore, be somewhat of an

artefact. On ellipsoid spores the angles are very slight, longitudinal ridges: on the globose spores there is no ridging, and I assume that the spores, on drying, become faintly polygonal. The small spores of *D. Vanderystii*, with but slightly thickened walls, do not show this marking. The character is useful to distinguish the amygdaliform spores of *Deflexula* from those of *Pterulicium*, which are not sulcate-angled.

Except for slight differences in the width of the hyphae, the microscopic structure is the same in all the species. The colour seems to be generally white, turning pale yellowish with age and, in some cases, suffused with lilac. The size and ramification of the fruit-body may be misleading because some species begin to spore on young, unbranched fruit-bodies, and it is possible that large and even ramified states of the small species occur, e.g. *D. subsimplex*. The new key, which I give, is based, therefore, primarily on spore-characters.

Several species develop very slender, short rhizomorphs from the tufts of fruit-bodies and these branch adventitiously to give rise to new 'spines', thus adding to the size and fasciculate appearance of the old tuft.

The best way of reviving dried fruit-bodies of this, and of other Pteruloid genera, is to immerse them in 3 per cent. potash, which quickly makes them uncurl and regain a more or less natural shape. They may then be scrutinized under the binocular microscope, when a regular form will appear out of the dried tangle.

The species grow on dead wood or bark and are probably widely distributed. Thus, *D. lilaceo-brunnea* occurs in tropical Asia and tropical America, and *D. subsimplex* occurs in tropical America, West Africa, the Philippine Islands, and the Bonin Islands (if *D. pacifica* is synonymous).

Deflexula emend.

Fruit-bodies with *down-growing branches*, either ramified with horizontal or deflexed axis and multifid-bifid decurved branches (usually with adventitious branchlets), or as small clusters of simple or sparingly and adventitiously branched, down-pointing spines, each cluster developing from an initial spine by adventitious proliferation of new spines from its base or from slender rhizomorphs.

Lignicolous or corticolous: 10 spp., pantropical and in temperate North and South America.

Spores *white, smooth or becoming sulcate-angled* (? when dried), mostly large, aguttate, slightly thick-walled.

Basidia large.

Microscopic structure as in *Pterula*.

KEY TO THE SPECIES

Spores more or less globose

Branched, -18 mm. long: sp. 9-11 μ *D. fascicularis*

Simple, -6 mm. long

Sp. 11-14 μ *D. lilaceo-brunnea*

Sp. 6 $\times$ 5 μ *D. Vanderystii*

Spores ellipsoid, usually sulcate-angled (when dried)

Branched (simple when young and small)

—28 mm. long, not secund: sp. 11-14 × 6-8 μ *D. subsimplex*

—65 mm. long: sp. 12-15 × 7-10 μ

Branches simple, secund on a long horizontal axis *D. pennata*

Branches not secund, multifid, bifid, or adventitiously

branched, the axis not prominent *D. major*

(Sp. 9-11 × 6 μ *Pterula secundiramea*)

Simple, —8 mm. long

Sp. 18-24 × 8-10 μ *D. mangiformis*

Sp. 14-16 × 10-12 μ *D. ulmi*

Sp. 11-14 × 8-11 μ *D. sulcispora*

(Sp. 18-20 × 10-12 μ : 1 mm. high *Pterula macrospora*)

Spores unknown *D. argentina*

***D. argentina* (Speg.) comb. nov.**

Basynym: *Mucronella argentina*, Speg., Fung. Arg. 1899, 178.

Examination of the type-specimen (Inst. Speg. 19272, Santa Catalina, Buenos Aires, July 16, 1889) shows that it is a typical *Deflexula*, but, unfortunately, as Spegazzini wrote on the packet 'spora non visae'. In hyphae and large basidia the species agrees with *D. lilaceo-brunnea* and, on one among eight fruit-bodies that I examined, I found a few globose, apiculate, spores 10-12 μ wide: they were too few, however, to be sure that they belonged to the fungus, especially in view of the many extraneous spores, as well as diatoms, on the hymenium. The species appears identical with *D. lilaceo-brunnea* var. *elongata*, but one must await its rediscovery (in the type-locality, if that exists) before one can decide. Spegazzini gave the colour as cinereous, which may refer to the dried specimen.

***D. fascicularis* (Bres. et Pat.) Corner**

Lloyd Cat. Nos. 32762, 32763 (Samoa).

Both collections have subglobose spores 9.5-11.5 μ wide, which are distinctly, if slightly, angled in optical section.

***D. lilaceo-brunnea* Corner**

var. *elongata* var. nov.

Receptacula longiora, ad 6 mm., haud lilaceo-tincta: ad lignum emortuum in silvis, Brazil (Rio de Janeiro, Corcovado, Corner 781-*typus*).

Fruit-bodies —6 × 0.2-0.5 mm., deflexed, fasciculate in small tufts, *simple* but proliferating from the base, rarely with an adventitious branch beyond the base, *white then pale ochraceous*, finally brownish at the base, never tinged lilac.

On dead wood in the forest: Brazil (Corner 781, Rio de Janeiro, Corcovado, c. 500 m. alt., Dec. 5, 1948: Rick s.n., São Leopoldo, Rio Grande do Sul, in U.S. Dept. Agr. Myc. Coll., as *Pterula subplumosa* P. Henn.: Lloyd Cat. No. 32722, Irg. Rick, Brazil, det. *P. incarnata* Pat.).

Spores 11–14 μ , globose, smooth or very slightly angled on drying, finely granular-guttulate when fresh, the apiculus 1.5 μ long.

Basidia 45–60 $\times$ 15–17 μ (living), strongly clavate from the narrow, 3 μ wide, base, 4-spored.

Hymenium slightly thickened, with paraphysoid hyphae 1.5–3 μ wide, blunt, thin-walled, flexuous, rarely septate, projecting $\sim$ 100 μ : cystidia absent.

Skeletal hyphae 3–5 μ wide: generative hyphae clamped.

This description is based on my Brazilian collection 781. The microscopical characters are the same as in the Malayan collection of *D. lilaceo-brunnea*, but the fruit-bodies of the Brazilian collections were nearly twice as long, and



FIG. 3. *Deflexula major*: Lloyd Cat. No. 32743. $\times$ 1: spores, $\times$ 1,000.

those of Corner 781 lacked the lilaceous colour. The difference in size may be one of age, but the Malayan specimens, the collection of which I clearly remember, gave no indication of growing larger. The identification of Rick's collection with *Pterula subplumosa* must be an error: another collection in U.S. Dept. Agr. Myc. Coll. (herb. Bresadola), named *P. subplumosa*, is *Pterula Uleana*. Lloyd Cat. No. 32722 has a few slight adventitious branches on some of the larger 'spines', as in young *D. fascicularis* (which has smaller spores).

***D. major* sp. nov.**

Fig. 3.

Receptacula usque 6.5 cm. longa, fasciculata, multum ramosa, plerumque ex inferiore: stipite ad 8 $\times$ 0.3–0.8 mm., glabro, ad basim paulum dilatato, ut videtur haud proliferanti: ramis multifidis dein bifidis, etiam ramulis adventitiis, apicibus filiformibus: ramis obliquis hymenio unilaterali et infero gerentibus: sporis 13–15 $\times$ 8–10 μ , amygdaliformibus vel lacrimiformibus, paulum sulcato-angulatis: hyphis skeletalibus 2–3.5 (–4) μ crassis: habitatione ignota, ex Africa (Belgian Congo, Lloyd Cat. No. 32743, leg. H. Vanderyst).

–6.5 cm. long, *fasciculate*, *much branched*, mainly to the lower side (colour?: brownish flesh-colour when dried): stem –8 $\times$ 0.3–0.8 mm., glabrous, the

base slightly expanded, apparently not proliferating: *branches multifid, then bifid, also with adventitious branchlets*, tapering to filiform tips, the hymenium unilateral on the lower side of oblique branches, absent from the stem.

Habitat?: Belgian Congo (Lloyd Cat. No. 32743, leg. Rev. Hyac. Vanderyst, Nov. 1922).

Spores $13-15 \times 8-10 \mu$, white, more or less *amygdaliform* or *lachrymiform*, *slightly sulcate-angled* with 6-8 angles in optical t.s., the wall *c.* 0.5μ thick, the prominent apiculus 1.5μ long.

Basidia *c.* $50 \times 14-16 \mu$: 4 sterigmata: cystidia none: hymenium apparently not thickening.

Skeletal hyphae $2-3.5 (-4) \mu$ thick, *narrow*, the walls $0.5-1 \mu$ thick: generative hyphae clamped.

This remarkable species was referred by Lloyd to his *Pterula penicellata*, but from the position of the hymenium, from the curvature of the branches, from the spores, and from the large basidia, it is clearly *Deflexula*. The spores are practically identical with those of *D. pennata*, but the skeletal hyphae are narrower and the fruit-body is of different form.

D. mangiformis sp. nov.

Receptaculis usque ad $7 \times 0.2-0.4$ mm., fasciculatis, simplicibus sed basi proliferantibus: sporis $18-24 \times 8-10 \mu$, magnis, fusiformibus, subacuminatis, 5-7 subangulato-subsulcatis: ad lignum emortuum, Panama, Rio Chiriqui Viejo, G. W. Martin 2102.

Receptacles $-7 \times 0.2-0.4$ mm., fasciculate in scattered clusters, simple but proliferating from the base, deflexed, acerose (? white, when fresh), pallid brownish-white when dried.

On dead wood: Panama (Prov. Chiriquí, Upper Rio Chiriquí Viejo, *c.* 1,700 m. alt., June 28, 1935, G. W. Martin 2102).

Spores $18-24 \times 8-10 \mu$, white, smooth, *fusiform*, *subacuminate*, 5-7-angled in optical t.s., the wall slightly thickened, finely granular opalescent, the apiculus 1μ long.

Basidia $60-70 \times 15-18 \mu$: sterigmata 4.

Hymenium not thickening: cystidia absent.

Skeletal hyphae 3-6 (-8) μ wide, the walls $1-2.5 \mu$ thick, sometimes branched: generative hyphae 3-5 μ wide, clamped: the surface of the lower part of the stem with many, narrow, sterile basidia with thickened walls ($1-2.5 \mu$), among sterile, short, emergent hyphal ends: granular crystalline matter abundant among the superficial hyphae.

Because of its very large, subacuminate spores I have described this as new, but it may be *Pterula macrospora* Pat. or merely a variant of *Deflexula sulcispota*.

D. pennata (P. Henn.) comb. nov.

Basinym: *Pterula pennata* P. Henn., Hedw. 1904, 174: Corner, Monogr., 1950, 536.

Fig. 4.

—5 cm. long, subfasciculate, monopodial, more or less horizontal, with short secund branches: *stem* 1 mm. wide, short, dividing into 2 (? more) tapering axes, 0.5 mm. wide below: *branches* —5 mm. long, adventitious, in one or two rows, simple or with a short branchlet, slightly curved, acerose.

On rotten wood: Brazil (Amazonas, Rio Jurua).

Spores $12-15.5 \times 7-9 \mu$, colourless, more or less amygdaliform, 5-8 sulcate angled, the wall slightly thickened, apiculus $0.7 \times 1 \mu$.

Basidia *c.* 14μ wide: hymenium apparently not thickening: cystidia none.

Skeletal hyphae $2.5-5 \mu$ wide, the walls $0.5-1 \mu$ thick: generative hyphae clamped.

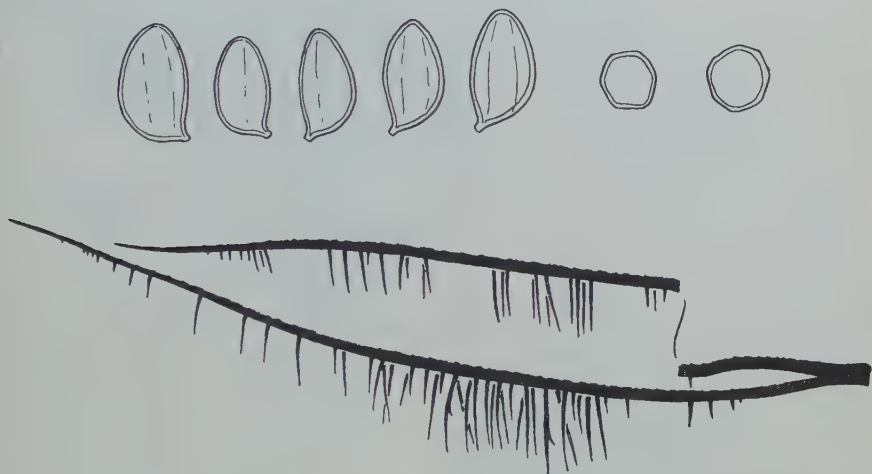


FIG. 4. *Deflexula pennata*: fruit-bodies. $\times 2\frac{1}{2}$: spores, $\times 1,000$.

Upside of the axes with tufts of generative hyphal branches, $-120 \times 2.5 \mu$, septate, with simple blunt ends, sterile.

This description is drawn from parts of the type-collection in the herbaria of Lloyd and Bresadola (U.S. Dept. Agr. Myc. Coll.), kindly loaned to me by Dr. Stevenson. My former interpretation of the species was erroneous. It is a true and very remarkable *Deflexula*, but whether *Pterula subplumosa* and *Hydnum Sprucei* are identical remains to be proven. Compare *Pterula secundiramea*.

D. subsimplex (P. Henn.) comb. nov.

Basynym: *Pterula subsimplex* P. Henn., Hedw. 1897, 197: Corner, Monogr., 1950, 522.

Synonyms: ? *Pterula importata* P. Henn., Verh. Bot. Ver. Prov. Brand. 40, 1898, 121, t.l., f.l.: Corner, Monogr. 1950, 539-40.

? *Pterula nivea* Pat., Bull. Soc. Myc. Fr., 18, 1902: Corner, Monogr., 1950, 398 (ut *Deflexula*).

? *Mucronella pacifica* Kobayashi, Bot. Mag. Tokyo 53, 1939, 160, f. 4: Corner, Monogr., 1950, 399 (ut *Deflexula*).



FIG. 5. *Deflexula subsimplex*: A, Rick s.n., 1906 (Herb. Bres.). B, Rick s.n., 1924: the remainder from Lloyd Cat. No. 42100. $\times 5$.

(*Hydnum fastigiatum* Rick *ms.*, *Lachnocladium mucronella* Rick *ms.*, *Pterula inclinata* Rick *ms.*, *P. ramosa* Rick *ms.*, *Clavaria mucronella* Bres. *ms.*) Figs. 5-8.

—28 mm. long, fasciculate, becoming rather stout, deflexed (? white or pale ochraceous, when fresh): stem $-8 \times 1-2$ mm., more or less horizontal, dividing into several deflexed branches, often proliferating new branches (many

abortive): *branches* 0.2–1 mm. wide below, simple or usually with several adventitious branchlets (mostly on the lower side), sometimes apparently multifid, the ends tapering and acute.

On dead wood and bark in the forest, apparently pantropical: *Brazil* (Rio Grande do Sul, pr. São Leopoldo, leg. J. Rick, as Lloyd Cat. Nos. 30227, 32718, 32720, 32757, 42086, 42100, 42109: *Bahia*, leg. C. Torrend, as Lloyd



FIG. 6. *Deflexula subsimplex*: Lloyd Cat. No. 32720. $\times 5$.

Cat. No. 42115: also Rick s.n., 1906, as *Pterula subsimplex*, Rick s.n., 1924, as *Pterula* sp., and Rick s.n., 1932, as *Pterula inclinata* ms., in U.S. Dept. Agr. Myc. Coll.): *Colombia* (U.S. Dept. Agr. Myc. Coll. s.n., leg. C. Garces O., Garzon, Huila, Nov. 1939, on bark of *Theobroma cacao*: G. W. Martin 3464, 3468, 3582, Dept. Magdalena, Sierra Nevada de Santa Marta, Hacienda Cincinnati, c. 1250 m. alt., Aug. 1935): *Guadeloupe* (as *Pterula nivea*): *Costa Rica* (U.S. Dept. Agr. Myc. Coll., A. M. Brenes 4942, La Balsa de San Ramon, Aug. 26, 1926): *Philippine Islands* (Lloyd Cat. No. 49939, J. Corrales 6597, Mt. Maquiling, Luzon, Oct. 26, 1919): *Bonin Islands* (as *Mucronella pacifica*).

Spores $10.5\text{--}14 \times 6\text{--}8.5 \mu$, white, ellipsoid-pruniform, with 4–5–6 slight longitudinal ridges, the wall distinctly thickened, the apiculus c. 1μ long.

Basidia $40-50 \times 11-15 \mu$ in dried specimens, clavate: sterigmata 4: cystidia none.

Skeletal hyphae $3-6$ (-7) μ wide, the walls $0.5-2 \mu$ thick: generative hyphae clamped.

var. **multifida** var. nov.

Fig. 7.

Ramis apicibus multifidis, usque 13 mm. longis, basim versus $0.2-0.7$ mm. latis: sporis $10-13 \times 6.5-7.5 \mu$: hyphis structilibus $3-5 \mu$ latis.

Ad corticem emortuum: Liberia (U.S. Dept. Agr. Myc. Coll., leg. O. F. Cook, Mt. Coffee, April 1894).



FIG. 7. *Deflexula subsimplex* var. *multifida*. $\times 5$.

I have named these collections on the authority of Bresadola, for the collection J. Rick s.n., 1906, in U.S. Dept. Agr. Myc. Coll., is from Bresadola's herbarium and so named by him. I have not been able to trace a type of *Pterula subsimplex*, but the original description, quoted in my monograph, agrees with young specimens of this fungus which seems to be common, at least in south Brazil: the small spores given by Hennings are probably those of a mould, common on dried specimens (as in the case of *Pterula Uleana*, which I will mention in a later paper). The mango-shaped subacute spores with slight longitudinal angles distinguish the species at once as a *Deflexula*,

and provide the readiest means of separation from *Pterulicium xylogenum* in the herbarium, for its spores are not so thick-walled and are not angled. Thus, it is likely that the collection from Uganda (Dummer 1185, in Herb. Kew.), referred in my monograph to *P. xylogenum*, belongs to *D. subsimplex*.

The fruit-bodies are very variable in appearance, according to their age and, perhaps, according to the substratum. Robust, branched specimens scarcely suggest the same species as the slender, simple ones, which may reach 18 mm. long: yet they are microscopically identical, and intermediate states

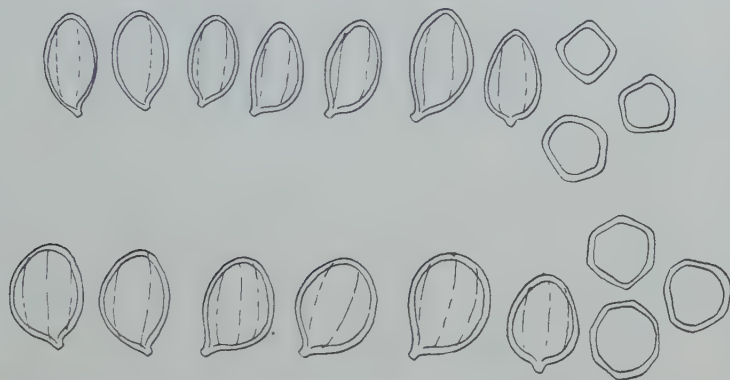


FIG. 8. Spores of *Deflexula subsimplex* above (Lloyd Cat. No. 32720), and of *D. sulcispora* below (Lloyd Cat. No. 32761).

can be found among Rick's copious collections, so that it seems that the simple states are young forms of the branched, the stem of which clearly thickens as a result of its adventitious branching. The most strikingly branched fruit-bodies occur in two collections made in Liberia by O. F. Cook, for which I have made the new variety. Cook, according to Dr. Stevenson, was in Liberia in 1892, 1894, and 1897 with the Colonization Society, before he joined the U.S. Dept. of Agriculture.

Compare *D. sulcispora* with wider, blunter spores and *D. mangiformis* with larger spores: both have, so far as known, simple fruit-bodies.

Occasionally it seems that the fruit-bodies may become thinly clothed with slender fascicles of hyphae $300\ \mu$ long, but this may be a result of enclosure in a tin for some hours, or days.

D. sulcispora sp. nov.

Figs. 8, 9.

Ut *D. subsimplex*, sed sic differt: receptaculis minoribus, $8.5 \times 0.15-0.3$ mm., simplicibus: sporis $10.3-13.5 \times 8-10.5\ \mu$, latioribus, pruniformibus, obtusis, 6-9-sulcato-angulatis: hyphis structilibus $2.5-4\ \mu$ latis, angustioribus: Brazil, Uganda: *typus*—Lloyd Cat. No. 32761, leg. J. Rick. ? pr. São Leopoldo, Rio Grande do Sul.

$8.5 \times 0.15-0.3$ mm., fastigate in clusters of 5-20, deflexed, simple,

proliferating new branches from the base, white then pale straw-yellow to pale brownish ochraceous, and finally suffused pale lilaceous from the base (Corner 779): in some cases developing a stalk $-1 \times 0.5-0.7$ mm., as a result of basal proliferation.

On dead fallen wood and bark in the forest: *Brazil* (Rio Grande do Sul, leg. J. Rick, Lloyd Cat. Nos. 32756, 32758, 32759, 32760, 32761, the last being the *type*: São Leopoldo, leg. J. Rick, U.S. Dept. Agr. Myc. Coll., as *Pterula crispa* Rick *ms.*: Bahia, leg. C. Torrend, Lloyd Cat. Nos. 32734, 32755, as *Pterula minuscula* Torr. *ms.*: Estado do Rio, Niteroi, Corner 779, Sept. 7, 1947): *Uganda* (Kipayo, Dummer 1450, April 1915, in Herb. Kew.).

Spores $10.3-14 \times 8-11 \mu$, white, *bluntly pruiniform*, more or less distinctly longitudinally sulcate with 6-9 rather faint angles, the wall distinctly thickened, finely granular opalescent when fresh, the apiculus $1-1.5 \mu$ long.

Basidia $35-46 \times 14-16.5 \mu$ (living, Corner 779), $35-40 \times 11.5-13.5 \mu$ in dried specimens: sterigmata 4, 7-9 μ long: hymenium not thickening: cystidia absent.



FIG. 9. *Deflexula sulcispora*: left, Lloyd Cat. No. 32761; right, Rick s.n. (São Leopoldo). $\times 5$.

Skeletal hyphae $2.5-4 \mu$ wide, rarely 4.5μ , narrow, the walls -1.5μ thick: generative hyphae clamped (except Corner 779 without clamps).

This species, which I have recognized from ten collections, comes very near to *D. subsimplex*, but the fruit-bodies appear always to be small and simple, though proliferating basally; the spores

are wider and blunter, with more angles, and the skeletal hyphae are narrower. It is probably pantropic.

D. Vanderystii (P. Henn.) comb. nov.

Fig. 10.

Basinym: *Pterula Vanderystii* P. Henn., Fl. du Bay- et Moy.-Congo 2, fasc. 2, 1907, 96: Corner, Monogr. 1950, 527.

$-3 \times 0.15-0.3$ mm., gregarious and fasciculate, deflexed, simple, but proliferating from the base and from short and slender rhizomorphs (-4×0.1 mm.).

On bark: Liberia (Mt. Coffee, April 1894, leg. O. F. Cook, s.n., U.S. Dept. Agr. Myc. Coll.).

Spores $5.5-6.5 \times 4.7-5.5 \mu$, white, subglobose to lachrymiform, smooth, the wall slightly thickened, not sulcate-angled, the apiculus -1μ long.

Skeletal hyphae $2.5-3.5$ (-4) μ wide, the walls $0.5-1 \mu$ thick: generative hyphae clamped.

This description is based on Cook's collection. The original was described from Kisantu on dead wood, as quoted in my monograph. I can see no

difference. The two collections are from the same geographical region and agree in the remarkably small spores, which were very abundant in the Liberian collection, as well as in the small size of the fruit-body. The spores are the smallest yet known in *Deflexula*.

However, in Bresadola's herbarium (U.S. Dept. Agr. Myc. Coll.) there is a minute fragment, consisting of two fasciculate fruit-bodies of *Deflexula*-form, which is labelled as the type collection without further detail. The fruit-bodies have narrow skeletal hyphae, as in Cook's Liberian specimens, but there are abundant spores $10-15 \times 7-9 \mu$ thin-walled, amygdaliform or lachrymiform, not sulcate-angled, and with an apiculus 1μ long. These spores, apparently belonging to the fungus, are so different from those described by Hennings ($5-6 \mu$, subglobose) that it is not improbable that two species have been mixed in the original collection or in Bresadola's herbarium.



FIG. 10. *Deflexula Vanderystii*, O. F. Cook s.n. (Liberia). $\times 5$: spores, $\times 1,000$.

PARAPTERULICIUM gen. nov.

As *Pterulicium* but with gloecystidia and either dichophyses or papillate skeletal hyphal ends in the hymenium: generative hyphae without clamp-connexions: resupinate corticioid patch with skeletal hyphae: fruit-bodies drying floccoso-coriaceous and light-coloured: on dead twigs and leaves in the forest, Brazil: 2 spp., typus—*P. subarbusculum*.

Ut *Pterulicium*, etiam receptaculo corticioideo evoluto, sed hymenio gloecystidiis et vel dichophysibus vel hyphis structilibus papillatis praedito: receptaculo corticioideo hyphis structilibus praedito: receptaculis siccis floccoso-coriaceis, pallidis: ad ramulos et folia emortua in silvis, Brazil.

The fruit-bodies of the two Brazilian species that I refer to this genus resemble those of *Pterula* with adventitious branching, but, instead of drying hard and horny, they become floccoso-coriaceous and remain light-coloured. They agree with *Pterulicium* in having a corticioid state, which is certainly fertile in *P. subarbusculum*. Microscopically they differ from all other *Pteruloid* fungi in the presence of gloecystidia. Further, both species lack clamps. But, between themselves, they show striking differences. In *P. subarbusculum* there are in the hymenium many small dichophyses with very narrow arms, and they arise from the generative hyphae of the subhymenium. In *P. octopodites* there are no dichophyses, but the very abundant, somewhat tapered, ends of the skeletal hyphae in the subhymenium are more or less profusely set on one side with one or two rows of short processes, for the most part as warts. The second difference concerns the walls of the hyphae which swell strongly in dilute potash in *P. octopodites*, so that the lumen may almost disappear, but which remain firm in *P. subarbusculum*. The differences suggest

two subgeneric, if not generic, states. The two kinds of sterile hymenial element recall strongly the two kinds of cystidium of the Pteruloid genus *Dimorphocystis* and suggest that it may have been derived from such as *Parapterulicium* with the gloecystidia becoming the thick-walled cystidia and the dichophyses or papillate hyphal ends becoming the lobed cystidia. The spores of *P. subarbusculum* resemble those of *D. laevis*, which also lacks clamps.

While gloecystidia and dichophyses have not been found in other Pteruloid fungi, they occur, also with the lack of clamps, in *Lachnocladium*. The doubt may arise whether *Parapterulicium* should not be referred to the *Lachnocladium*-series. However, the colourless, dimitic hyphae, the adventitious branching, the corticioid patch, the form of the fruit-body, and the size and shape of the spores conform with the Pteruloid series: macroscopically the two species would be referred unhesitatingly to *Pterula*. The parallelism is further emphasized by the fact that the dichophyses are hymenial in *P. subarbusculum*, not cortical as in *Lachnocladium*, and that the gloecystidia of *Parapterulicium* are multiguttulate.

As in *Pterulicium*, the resupinate fructifications develop only on the under-side of the substratum. They may bear clavarioid fructifications, which curve round and grow upward to become fertile. The hyphae are slightly narrower than in the erect fructifications and are dimitic, skeletal being absent from the resupinate fructifications of *Pterulicium*. If these fructifications occurred without the clavarioid state, they would certainly be mistaken for '*Corticium*': indeed, dichophyses and such papillate hyphae occur in resupinate genera, as suggestive derivatives.

KEY TO THE SPECIES

- | | |
|--|-------------------------|
| With hymenial dichophyses: skeletal hyphal walls not swelling in dilute potash: solitary or gregarious | <i>P. subarbusculum</i> |
| With papillate skeletal hyphal ends in the hymenium, without dichophyses: skeletal hyphal walls swelling strongly in dilute potash: more or less fasciculate | <i>P. octopodites</i> |

***P. octopodites* sp. nov.**

Figs. 11, 12.

Receptacula usque 7 cm. alta, saepe fasciculata, ut in *P. subarbusculo*, ramulis saepe numerosis: hymenio hyphis structilibus unilateraliter papillois, dichophysibus deficientibus: sporis?: tunicis hypharum structilium in dil. potassio hydroxido tumescentibus: ad ramulos emortuos in silvis, Brazil (leg. J. Rick, typus—Lloyd Cat. No. 57704).

—7 cm. high, fasciculate, sparingly to rather profusely branched, generally bushier and more fasciculate than *P. subarbusculum*, drying pallid cream flesh-colour and floccoso-coriaceous (not horny), (? white when fresh), developing a resupinate patch —4 cm. long: *stem* more or less distinct, 0.5–1 mm. wide,

rarely branched from the base, attached by a thin whitish mycelial disc 1–3 mm. wide on the upper side of the substratum, or upcurving from the resupinate patch: *branches* 0.3–0.5 mm. wide below, adventitious, tapering to



FIG. 11. *Parapterulicium octopodites*. $\times 2$.

filiform tips, simple or with obliquely ascending branchlets to the 2nd–4th degree, often spuriously multifid.

On the bark of fallen twigs and branches in the forest: Brazil (Rio Grande do Sul, pr. São Leopoldo, leg. J. Rick, Lloyd Cat. Nos. 30225, 30226, 32740B, and 57704 *typus*).

Spores and basidia?

Gloeocystidia as in *P. subarbusculum*, often acute or acuminate, abundant on all parts of the fruit-body from the base of the stem upwards.

Dichophyses absent, but the abundant endings of the skeletal hyphae ($2\text{--}3.5\ \mu$ wide) in the hymenium set on one side with one or two rows of short, blunt, mostly simple, protuberances $2\text{--}10 \times 1\text{--}2.5\ \mu$.

Skeletal hyphae $5\text{--}9.5\ \mu$ wide in dilute potash, the walls swelling strongly and almost obliterating the lumen, occasionally branched, unlimited: generative hyphae $2.5\text{--}6\ \mu$ wide, septate, often closely, without clamps.

Resupinate patch with skeletal hyphae and hymenial gloeocystidia.



FIG. 12. *Parapterulicium octopodites*: the ends of skeletal hyphae in the hymenium. $\times 1,000$.

All four collections are sterile, the hymenium having at most incipient basidia. The fruit-bodies of Pteruloid fungi usually develop slowly and the temptation is to collect them before they have had time to mature. The tuberculate or papillate skeletal hyphal ends of the hymenium, resembling under the microscope the arms of an octopus, do not occur in other clavarioid fungi, though common enough on the stem or pileus of agarics. The swelling of the hyphal walls in dilute potash is the readiest distinction from the next species.

***P. subarbusculum* sp. nov.**

Figs. 13, 14, 15.

Receptacula ad 6 cm. alta, ramosa, monopodialia, alba, dein sordide incarnata, mycelio disciformi parvo floccoso, ad 2.5 mm. lato, affixa: ramis simplicibus vel semel ramosis, adventitiis, ad $18 \times 0.1\text{--}0.2$ mm., apicibus filiformibus: sporis $11\text{--}15 \times 2.5\text{--}3\ \mu$, albis, levibus, elongatis, aguttatis: hymenio ad $80\ \mu$ incrassato: gloeocystidiis ad $80 \times 9\text{--}15\ \mu$, clavatis vel subcylindricis, multiguttulatis, tenuiter tunicatis, immersis: dichophysibus $15\text{--}30\ \mu$ latis, hyalinis, crasse tunicatis, in hymenio et subhymenio (haud in carne proprio nec in medulla receptaculi), ramis ter-sexiens dichotomis, basi $1\text{--}2\ \mu$ latis, apicibus filiformibus et vix $0.5\ \mu$ latis, attenuatis: hyphis structilibus $4\text{--}7\ \mu$ latis: hyphis tenuiter tunicatis $3\text{--}7\ \mu$ latis, haud septato-fibulatis: Corner 780 (Rio de Janeiro, Corcovado, pr. Paineiras, Nov. 24, 1948).

Fruit-bodies to 6 cm. high, solitary or in small groups, gregarious, *monaxial with adventitious branches to the first or second order, rarely the third, white, then drab flesh-colour*, drying pale fawn flesh-colour with the stem somewhat cinnamon brown: stem $-25 \times 0.3-0.5$ mm., glabrous or adpressedly sub-



FIG. 13. *Parapterulicium subarbusculum*. $\times 2$.

fibrillose, sterile, becoming pale brownish, *attached by a small floccose mycelial disc* $1.5-2.5$ mm. wide: *branches slender*, $-18 \times 0.1-0.2$ mm. at the base, tapering to filiform points, mostly unbranched, spreading widely: flesh rather tough.

On dead twigs, petioles and leaves in the forest: Brazil (Rio de Janeiro, Corcovado, pr. Paineiras, c. 500 m. alt., Nov. 24, 1948, Corner 780).

Spores $11-15 \times 2.5-3 \mu$, white, smooth, long, narrow, elongate pip-shaped, the apex obtuse or subacute, the base acute, straight or subsigmoid, aguttate, the apiculus very small.

Basidia $25-30 \times 5-6 \mu$, subclavate: sterigmata 4, $6-7 \mu$ long.

Gloeocystidia $-80 \times 9-15 \mu$, clavate or cylindric, blunt, thin-walled, with dense multiguttulate or granular-guttulate contents, extending through the

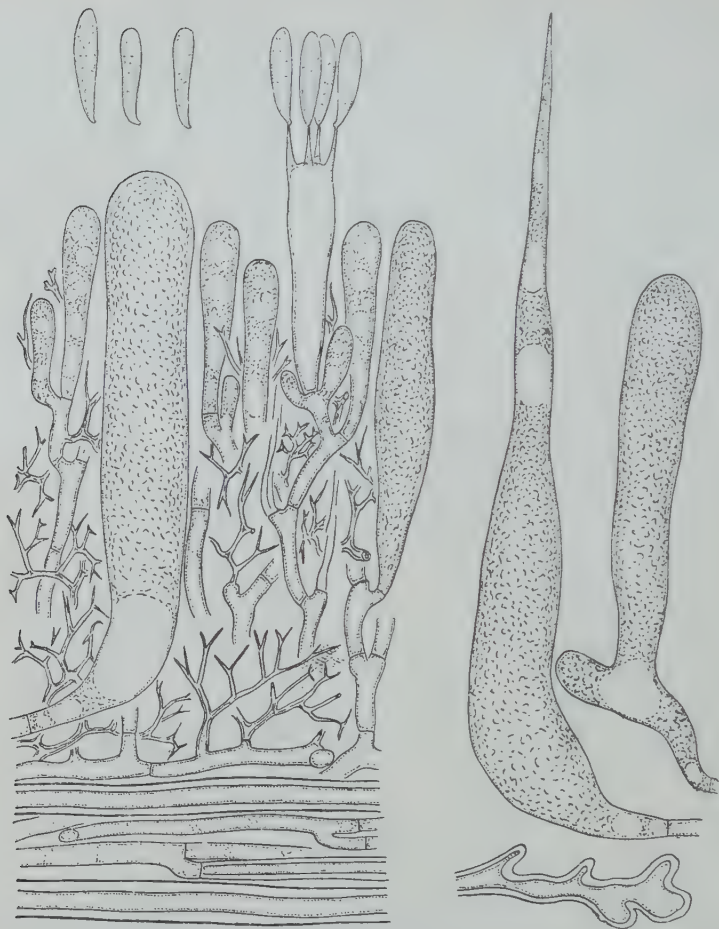


FIG. 14. *Parapterulicium subarbusculum*: hymenium, the ending of a skeletal hypha in the subhymenium, and (right) gloeocystidia from the stem. $\times 1,000$.

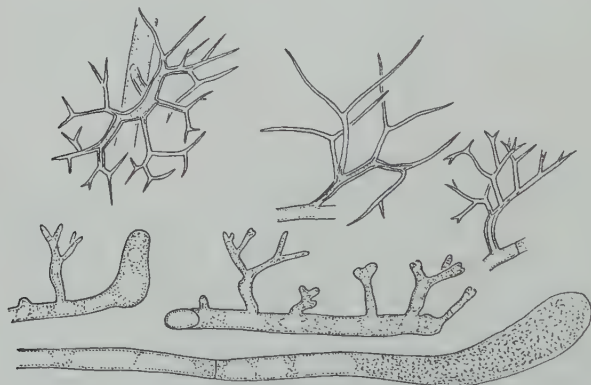


FIG. 15. *Parapterulicium subarbusculum*: developing dichophyses and a young gloeocystidium. $\times 1,000$.

thickened hymenium, scarcely protruding, arising primarily from the longitudinal subhymenial hyphae and often with a short longitudinal stalk, secondarily arising from the subhymenial hyphae of the thickening hymenium, occasionally forked: on the stem, many gloeocystidia with elongate filiform tips $\sim 100\ \mu$ long, and tapering to $0.5\text{--}2\ \mu$ wide at the apex.

Dichophyses $15\text{--}30\ \mu$ wide, colourless, very slender, abundant in the subhymenium and thickened hymenium, with a short, slightly thick-walled stalk, $3\text{--}15 \times 1\text{--}2\ \mu$, and tapering arms 3–6 times dichotomous, ending in filiform, straight or flexuous, tips less than $0.5\ \mu$ wide, arising from the subhymenial generative hyphae.

Hymenium present on the branches and the upper part of the axis of the fruit-body, absent from the stem, thickening to $80\ \mu$, the basidia eventually forming a fairly compact layer: subhymenial hyphae $1.5\text{--}2.5\ \mu$ wide, with submucilaginous walls, developing dichophyses, gloeocystidia and basidia: sterile hymenium on the stem consisting of scattered abortive basidia among dichophyses and gloeocystidia, the walls of the dichophyses and superficial hyphae becoming brownish, not sclerotoid.

Hyphae dimitic: generative hyphae $3\text{--}7\ \mu$ wide, stout, cylindric, *without clamps*, the walls thin or very slightly thickened: skeletal hyphae $4\text{--}7\ \mu$ wide, the walls $1\text{--}2\ \mu$ thick, aseptate, colourless, stout, regular, unlimited, rarely branched, sometimes with lobed apex in the subhymenium: hyphal tips at the growing apices $1.5\text{--}2.5\ \mu$ wide: mycelial hyphae $1.5\text{--}2.5\ \mu$ wide, thin-walled, without clamps.

Corticoid patch on the underside of twigs and petioles, *c.* $50\ \mu$ thick, ~ 1.5 cm. wide, white or pale pinkish flesh-colour, pruinose, separable, composed of a narrow, loose (not agglutinated), basal layer of generative hyphae $2\text{--}4\ \mu$ wide, generally with rather few, narrow, flexuous, and not infrequently branched, skeletal hyphae $3\text{--}4\ \mu$ wide: basidia, spores, dichophyses, and gloeocystidia as on the erect fruit-bodies.

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The Use of Random and Contiguous Quadrats in the Study of the Structure of Plant Communities

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With six Figures in the Text

ABSTRACT

Artificial 'communities' of discs have been constructed and sampled by random throws of quadrats of different sizes and by a grid of contiguous quadrats.

The use of a grid opens the way to more detailed study of the nature of the non-random distribution of species in the field, especially to the detection of mosaic patterns not normally revealed by subjective methods.

INTRODUCTION

THE use of quadrats thrown at random was originally developed, mainly by Scandinavian ecologists, in an attempt to obtain quantitative data on the abundance of species in plant communities. Results were commonly expressed in the form of percentage of quadrats occupied by the species. This percentage was assumed to be proportional to abundance. A number of writers have pointed out on theoretical grounds that the relationship is not a linear one (see Ashby, 1935). Even so, if the distribution of individuals is random, it is possible to calculate abundance from the percentage of quadrats not containing the species. A more serious difficulty is the non-random distribution of many species. If the distribution of a species is random the proportions of quadrats containing 0, 1, 2, 3, . . . individuals will fall on a Poisson series

$$e^{-m} \left(1, m, \frac{m^2}{2!}, \frac{m^3}{3!}, \dots \right),$$

where m is the mean number of individuals per quadrat. A number of observers have tested data obtained from randomly thrown quadrats for fit to Poisson expectation. In some cases species in semi-natural vegetation have been found to have a random or nearly random distribution, e.g. in various examples of grassland examined by Blackman (1935, 1942). More generally, however, species are found to be overdispersed, i.e. individuals tend to be aggregated (Ashby, 1948). A particularly striking example is furnished by Clapham (1936), who examined data compiled by Steiger for prairie vegetation and showed that of 25 species in 'low prairie' only 4 species were randomly distributed and of 19 species in 'high prairie' none was random. Further,

Blackman (1942) has shown that the type of distribution may vary for any one species. The realization of these difficulties has made it clear that the use of random quadrats in the determination of abundance is strictly limited. Archibald (1948, 1950) has recently shown that the observed distribution of species showing overdispersion may frequently be described by Neyman's Contagious series or by Thomas's Double Poisson series. Both series are based on the assumption that the occurrence of one individual in an area increases the chance of other individuals being present. In such cases abundance can be estimated from the number of quadrats containing no and one individual respectively.

Since the limitations of the method for determination of abundance have been realized attention has been directed rather to the nature and degree of non-randomness. Ashby (1948) has pointed out that, while in a large sample area non-randomness is to be expected, due to heterogeneity of soil and microclimate, overdispersion is commonly found even in small areas and in apparently uniform habitats. Such overdispersion can in some cases be explained by the biological characteristics of the species concerned, e.g. by occurrence of vegetative spread or production of heavy seeds. There remains, however, a residuum of cases where there is no obvious explanation.

Watt (1947*a*) has brought forward evidence to show that in some communities which are apparently in equilibrium with their environment the equilibrium is a dynamic one. At any one place in a stand of a community the vegetation passes through a cycle of phases. At any one time different areas will be at different stages so that a stand of the community has a mosaic pattern made up of the different phases. In the communities discussed by Watt the differences between the phases are apparent as differences in the specific composition, e.g. in the *Sphagnum* pool and hummock cycle, or in the stage of morphological development of the dominant or principal species, e.g. in the cycle shown by *Pteridium aquilinum* in Breckland. In some cases there is no detectable difference in the physical environment of the phases and the differences between them must be attributed to intrinsic properties of the species concerned. In other cases there are differences in, for example, nutrient level in the soil, which are correlated with the vegetation phases and seem more readily explicable as results of the latter, rather than vice versa. If, as Watt suggests, this cyclic change is a widespread phenomenon in plant communities, it is possible that the differences between phases may take the form of differences in the density of species. In this case the detection of the mosaic pattern will present greater difficulty and it is worth considering whether the non-randomness of distribution hitherto unexplained may be the expression of an unrecognized mosaic pattern, with the different phases having the same species at different densities but distributed at random within the areas of any one phase.

The size of the individual plant, or shoot in rhizomatous species, will set a lower limit to the size of the mosaic unit but, in a uniform environment, there is no theoretical upper limit. One of the difficulties in interpreting non-

randomness is the small size of the sample areas in which it can be detected. Blackman (1942) found overdispersion in grassland plots 18 by 15 ft. Archibald (1948) detected overdispersion in sample areas of 100 or 500 contiguous 20 sq. cm. quadrats on salt-marsh and chalk grassland. (The shape of the whole sample area is not stated.) Here the size of sample area is not very small compared with that of individuals, although unlikely to include much soil or microclimatic heterogeneity in the vegetation studied. Watt (1947*b*), however, found it possible to assign plots 1 ft. square to a phase with so large a plant as *Pteridium aquilinum*.

It is not immediately apparent what the expectation of number of quadrats containing 0, 1, 2, 3, . . . individuals will be if there is a mosaic of phases each containing the same species randomly distributed but at different densities in the different phases. It seemed worthwhile therefore to construct artificial 'communities' with discs and to test empirically the distribution obtained with random throws of a quadrat.

At the same time the opportunity has been taken to test another approach to the detection and interpretation of non-randomness. The writer became interested in this when working in tropical forest in the West Indies in 1948. It was desired to test the randomness or otherwise of the distribution of various tree species. The use of random quadrats has certain practical difficulties in such vegetation and it was necessary to use a lattice of contiguous quadrats. Although such quadrats are not randomly placed it is permissible to test the randomness or otherwise of individuals by comparing the counts obtained with Poisson expectation, because the null hypothesis presupposes the complete independence of individuals from one another. If, however, a non-random distribution is indicated by the test, i.e. individuals are not independent of one another, it is not obvious what conclusions may be drawn from the degree of departure from expectation. Against this disadvantage there is the advantage that, by the use of a row-and-column analysis of variance, it is possible to detect any unidirectional variation in density, e.g. up a slope, and, if there is any mosaic variation in density, to obtain some idea of the unit of heterogeneity by associating the quadrats into successively larger blocks in an analysis of variance, testing the different block variances against the residual variance within the smallest block size. Further, the residual variance itself may be used to test for non-randomness other than that due to larger-scale heterogeneity. The forest data obtained in this manner will be discussed in a later paper. The remainder of this paper is concerned with an account of the data obtained from artificial 'communities'.

METHODS

The artificial communities consisted of discs which were laid out on a floor on which a rectangle 3.2 by 1.6 m., divided into a grid of 512 units, each 10 cm. square, had been marked out.

Six layouts were used. These are considered in detail below, but it is convenient to discuss here the method of randomizing discs. It is not easy to

obtain a completely random arrangement. The possibility of determining the position of individual discs by co-ordinates taken at random by any of the usual methods was considered. This is undoubtedly the most reliable method but is extremely laborious and a quicker though somewhat less reliable method based on scattering by hand was used. (Such a method has been used in this department with satisfactory results for demonstrating to students Poisson distribution in random arrangements.) The operator walked up and down the grid area dropping discs two or three at a time until the required number had been dropped. Care was taken to walk along the edges of the grid area as well as in the centre so that a number of discs fell outside it. These were then picked up and the process repeated until all discs were lying in the grid area. As will be shown below, there is reason to believe that a slight constant bias was introduced by this procedure, but on the whole good fits to expectation for distributions supposedly random were obtained.

In each experiment the layout was sampled by 100 throws of each of seven sizes of square quadrats, having sides of length 10, 15, 20, 25, 30, 35, and 40 cm. respectively. The distribution of discs on the grid was also recorded. Before sampling commenced the discs were fixed in position by drops of glycerine jelly to prevent accidental movement.

Counts may be tested for fit to Poisson expectation in two different ways. Blackman (1935) calculated the appropriate Poisson series and tested the observed figures against it by means of a χ^2 test. This test has the disadvantage where the mean number of individuals per quadrat is low that the expected proportion falls very rapidly along the series and unless a large number of counts is made only the first two or three terms will have an expected value greater than five (generally considered the lowest expectation permissible in a χ^2 test), e.g. for 100 throws with a mean of 0.14 individuals per quadrat the expectation is

Individuals per quadrat	.	.	.	0	1	2 and over
Number of quadrats	.	.	.	87.0	12.2	0.8

and the expectation for all occupied quadrats must be lumped. In this case a χ^2 test cannot be employed as the one degree of freedom is used in estimating the mean. It might be argued that in the experimental layouts considered here the true mean is known independently of the data from randomly thrown quadrats, and might be used for calculating the Poisson series. This is so, but the mean estimated from the throws recorded has been used as being more comparable with field conditions.

Clapham (1936) used the relative variance (variance/mean) as a measure of departure from Poisson expectation, though testing the significance by a χ^2 test. In a Poisson distribution the variance is equal to the mean, so that a value for the relative variance greater than 1 indicates overdispersion and less than 1 underdispersion. The standard error of this ratio may be used to test the significance of its deviation from 1. Blackman (1942) obtained a value of the standard error from the formula

$$s = \sqrt{\left(\frac{2n}{(n-1)^2}\right)},$$

where n is the number of observations. Professor M. S. Bartlett (personal communication) states that it is not clear how this formula is derived and considers a more correct formula to be

$$s = \sqrt{\left(\frac{2}{n-1}\right)}.$$

The values given by Bartlett's formula have been used in this paper.¹ Provided the number of observations is reasonably large they do not differ greatly from those given by Blackman's formula.

Blackman found cases in the field where there was no significant overdispersion or underdispersion as measured by the relative variance, but where skewing of the distribution resulted in a significant deviation from expectation when the χ^2 test was applied. Both tests have been used on the data considered here. The discrepancies observed will be discussed later.

In addition to testing the grid data for fit to Poisson expectation an analysis of variance has been made in each case, in which variance attributed to blocks of 2, 4, 8, 16, &c., contiguous grid units is tested against the residual variance within the smallest block size. From the residual variance a 'corrected value' (residual variance/mean) of the relative variance may be obtained, testing randomness of individuals within the smallest block size. The standard error of this corrected value has been determined by Mr. J. H. Darwin and is given approximately by the formula

$$s = \sqrt{\left(\frac{2}{N}\right)},$$

where N is the number of degrees of freedom attributed to the residual variance.²

An objection which might be raised to the use in this way of an analysis of variance is that the tests of significance by the F (variance ratio) test may not be exact in view of the low values of the numbers recorded for individual grid units (including zero readings). Low values only occur, however, where the number of degrees of freedom available are large and this consideration renders the tests of significance reasonably reliable. On the other hand, it should be noted that for the largest groupings considerable fluctuations in the mean square may be expected in view of the small number of degrees of freedom available.

EXPERIMENTS

A completely random and three different types of non-random arrangement were used, two of the latter at different density levels. Details are given overleaf.

¹ For 100 observations $s = 0.1421$, for 512 observations $s = 0.06256$.

² In the analysis used $N = 256$ and $s = 0.08838$.

Experiment 1

This layout contained 125 discs arranged at random and was included principally to test the efficiency of the randomizing technique before preparing further layouts.

Experiments 2 and 3

These were designed as clumped arrangements such as have commonly been assumed to be indicated by overdispersion. In expt. 2, 25 discs were laid at random; 100 further discs were associated with these centres. The number

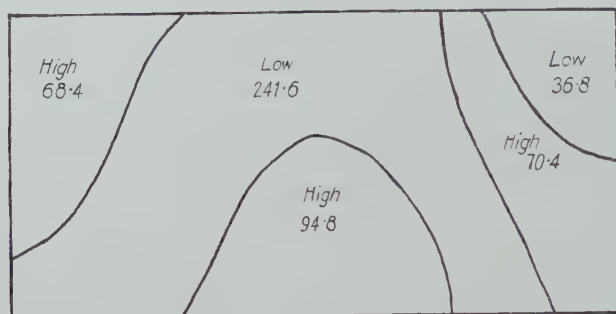


FIG. 1. Layout of experiments 4 and 5. Areas in grid units (of 100 cm.²).

of centres to receive 0, 1, 2, 3, . . . , &c., additional discs was calculated from a Poisson series with a mean of 4. The actual position of the additional discs was at random within a distance of 10 cm. from the corresponding centre.¹ A layout of clumps with a mean of five individuals per clump was thus obtained. In expt. 3, 50 centres and 75 associated discs were used, giving a less markedly clumped arrangement with the same mean number of individuals per unit area.

Experiments 4 and 5

These layouts represent one type of mosaic of high and low density areas of the kind postulated above. The areas of high and low density were comparatively large compared with the total area and more or less irregular in shape (see Fig. 1). The pattern was drawn on the grid area and the area of the patches measured by copying on to graph paper and use of a planimeter. In expt. 4, 100 green discs and 150 yellow discs were scattered over the grid area in the normal way. All green discs were then removed from the parts marked 'high density' in Fig. 1 and all yellow discs from those marked 'low density', leaving 52 green and 58 yellow discs localized in the 'low' and 'high' density areas respectively. The mean density of the green discs as at first scattered was 0.1953 per grid unit and of the yellow 0.2930 per grid unit. The

¹ This was achieved by placing a card cylinder 20 cm. in diameter symmetrically over each centre and dropping the associated discs into it.

density within their areas of green and yellow discs remaining was 0.1868 and 0.2483 per grid unit respectively. In this layout the difference in density between high and low density areas was comparatively small. In expt. 5 a larger difference in density was used on the same pattern of areas. Fifty green and 200 yellow discs were scattered (means of 0.0977 and 0.3906 per grid unit respectively) and there were finally 29 green and 95 yellow discs (means within their areas of 0.1042 and 0.4067 per grid unit respectively).

Experiment 6

This layout also represented a mosaic of areas of differing density, but one of smaller and more regular units (see Fig. 2). Three density levels were used,

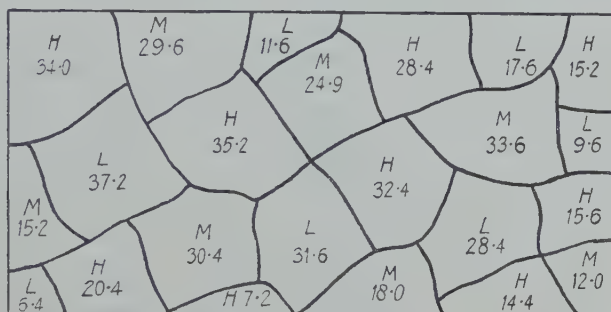


FIG. 2. Layout of expt. 6. H, high density; M, medium density; L, low density. Areas in grid units (of 100 cm.²).

50 green, 200 yellow, and 350 blue discs being scattered over the grid area. All except green discs were then removed from the parts marked 'low density' in Fig. 2, all except yellow from those marked 'medium density', and all except blue from those marked 'high density', leaving 16 green, 64 yellow, and 141 blue discs which had means within their areas of 0.1124, 0.3837, and 0.6953 discs per grid unit respectively.

RESULTS

Random layouts

Three sets of data from layouts presumed to be random are available, from expt. 1 and from the centre discs of expts. 2 and 3, having means of 0.2441, 0.0488, and 0.0976 discs per grid unit respectively. The results of relative variance and χ^2 tests and the analysis of variance of the grid data for these are given in Table I. They call for little comment. The four blocks of 128 grid units in expt. 1 have a significantly high variance. The numbers of discs actually recorded in the four blocks are, in order from one end of the area to the other, 24, 44, 37, 20; and it is clear that an unduly high proportion of the discs were laid in the centre of the area, i.e. the layout was not a completely random one. The results for 35-cm. quadrats in expt. 1, for 30- and 40-cm. quadrats in expt. 2, and for 30-cm. quadrats in expt. 3 show a significant

TABLE I
Random Distributions(a) 125 discs per 51,200 cm.² (Experiment 1)

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t.</i>	<i>P.</i>	χ^2 .	<i>n.</i>	<i>P.</i>
10 cm.	0.27	0.27	0.9618	0.27	0.7-0.8	—	—	—
15 cm.	0.60	0.27	1.1448	1.02	0.3-0.4	3.39	1	0.05-0.1
20 cm.	1.26	0.31	0.8757	0.88	0.3-0.4	0.73	2	0.5-0.7
25 cm.	1.86	0.30	0.8691	0.92	0.3-0.4	1.11	3	0.7-0.8
30 cm.	2.70	0.30	0.8417	1.11	0.2-0.3	4.42	5	0.3-0.5
35 cm.	3.25	0.27	0.8353	1.16	0.2-0.3	11.90	4	0.01-0.02
40 cm.	4.49	0.28	0.8616	0.97	0.3-0.4	10.79	6	0.05-0.1
Grid	0.24	0.24	1.0621	0.99	0.3-0.4	1.77	1	0.1-0.2

Analysis of variance of grid

			S.S.	<i>n.</i>	M.S.	<i>F.</i>	<i>P.</i>
Blocks of 256 grid units	.	.	0.23633	1	0.2363	—	—
" 128	"	.	2.69141	2	1.3457	4.82	0.001-0.01
" 64	"	.	1.00781	4	0.2520	—	—
" 32	"	.	2.20312	8	0.2754	—	—
" 16	"	.	2.65625	16	0.1660	—	—
" 8	"	.	7.5625	32	0.2363	—	—
" 4	"	.	16.375	64	0.2559	—	—
" 2	"	.	28.25	128	0.2207	—	—
" 1	"	.	71.5	256	0.2793	—	—

Corrected relative variance: 1.1442, *t* 1.63, *P* 0.1-0.2(b) 50 discs per 51,200 cm.² (Experiment 3)

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t.</i>	<i>P.</i>	χ^2 .	<i>n.</i>	<i>P.</i>
10 cm.	0.17	0.17	0.8384	1.14	0.2-0.3	—	—	—
15 cm.	0.25	0.11	0.9192	0.57	0.5-0.6	—	—	—
20 cm.	0.54	0.13	0.9510	0.34	0.7-0.8	0.07	1	0.7-0.8
25 cm.	0.82	0.13	0.9455	0.38	0.6-0.7	0.55	2	0.7-0.8
30 cm.	1.24	0.14	0.8166	1.28	0.2-0.3	11.38	2	0.001-0.01
35 cm.	1.60	0.13	0.9470	0.37	0.7-0.8	2.02	3	0.5-0.7
40 cm.	2.09	0.13	0.8032	1.38	0.1-0.2	2.42	4	0.5-0.7
Grid	0.10	0.10	0.9761	0.38	0.6-0.7	—	—	—

Analysis of variance of grid

			S.S.	<i>n.</i>	M.S.	<i>F.</i>	<i>P.</i>
Blocks of 256 grid units	.	.	0.07031	1	0.0703	—	—
" 128	"	.	0.39062	2	0.1953	2.08	0.05-0.2
" 64	"	.	0.0625	4	0.0156	—	—
" 32	"	.	0.84375	8	0.1055	1.12	> 0.2
" 16	"	.	1.5	16	0.0937	—	—
" 8	"	.	2.5	32	0.0781	—	—
" 4	"	.	6.75	64	0.1055	1.12	> 0.05
" 2	"	.	13	128	0.1016	1.08	> 0.05
" 1	"	.	24	256	0.0937	—	—

Corrected relative variance: 0.9600, *t* 0.45, *P* 0.6-0.7

(c) 25 discs per 51,200 cm.² (Experiment 2)

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t</i> .	<i>P</i> .	χ^2 .	<i>n</i> .	<i>P</i> .
10 cm.	0.07	0.07	0.9394	0.43	0.6-0.7	—	—	—
15 cm.	0.14	0.06	1.1573	1.11	0.2-0.3	—	—	—
20 cm.	0.36	0.09	0.8709	0.91	0.3-0.4	0.62	1	0.3-0.4
25 cm.	0.51	0.08	0.8515	1.05	0.2-0.3	0.27	1	0.5-0.7
30 cm.	0.62	0.07	0.7097	2.04	0.02-0.05	6.69	1	0.001-0.01
35 cm.	0.92	0.08	0.9811	0.13	0.8-0.9	0.11	2	0.5-0.7
40 cm.	1.14	0.07	0.6560	2.42	0.01-0.02	15.93	2	< 0.001
Grid	0.05	0.05	1.0331	0.53	0.5-0.6	—	—	—

Analysis of variance of grid

			S.S.	<i>n</i> .	M.S.	<i>F</i> .	<i>P</i> .
Blocks of 256 grid units	.	.	0.01758	1	0.0176	—	—
" 128	"	.	0.25391	2	0.1270	2.60	0.05-0.2
" 64	"	.	0.11719	4	0.0293	—	—
" 32	"	.	0.48437	8	0.0605	1.24	> 0.2
" 16	"	.	0.71875	16	0.0449	—	—
" 8	"	.	1.3125	32	0.0410	—	—
" 4	"	.	3.125	64	0.0488	—	—
" 2	"	.	6.25	128	0.0488	—	—
" 1	"	.	13.5	256	0.0488	—	—

Corrected relative variance: 1.0000, *t* 0, *P* 1

departure from expectation (for the 35-cm. quadrat in expt. 1 and the 30-cm. quadrat in expt. 3 only when tested by the χ^2 test). This may again be attributed to slight imperfections in the randomizing technique; though it must be remembered that, out of the 24 sets of counts considered, slightly over one aberrant result may be expected if a 5 per cent. probability level is taken as significant. Arising from these discrepancies there is some indication that a comparatively large quadrat size giving a mean approaching 1 and an expected percentage absence of less than 50 may provide a more sensitive test of randomness. This appears to fit with Bartlett's conclusion (appendix to Blackman, 1935; see also Bartlett, 1948) that the most efficient size of quadrat for density determination of a random arrangement has a mean of about 1.6, giving about 20 per cent. absence. The tendency in the field has hitherto been to aim at a rather larger percentage absence in selecting quadrat size.

One other point not directly concerned with tests for randomness must be mentioned here. In the table the means per quadrat have also been expressed as means per unit area (of 1 grid unit = 100 cm.²). It will be seen that in all cases this mean is higher than the true value. This feature has appeared throughout the experiments; the explanation was not immediately obvious, but it can be explained by the technique used. It is believed that in laying the discs, in spite of the care taken to include the margins of the area equally with the centre, more discs were in fact laid in the centre. In throwing the quadrats care was taken to throw them all over the area (so that in many cases they fell outside and had to be rethrown). In spite of this it appears that more samples

were taken from the centre than from the margins. This effect would be accentuated, particularly with the larger quadrats, by the necessity of ignoring throws where the quadrat fell partly outside the area.¹ These effects taken together would result in slightly higher means than the true mean for the area (as given for the grid), i.e. the effect was to sample principally a slightly smaller area with a slightly higher density than the one intended. This inaccuracy of layout may well be the non-randomness in the data just considered. The sampling of a smaller area would not of course in itself give an aberrant result if the layout were perfectly random. Since the effect was present throughout the experiments it is believed not to affect the comparison of different types of layout. It does, however, provide an additional reason for using the estimated rather than the true mean for calculating Poisson series.

Clumped distributions (Experiments 2 and 3)

Results for the total number of discs (centre and associated) in the clumped layouts of expts. 2 and 3 are shown in Table II. As might be expected, throws with all sizes of quadrats and the data from single units of the grid show a very highly significant departure from Poisson expectation by either test, with very

TABLE II
Clumped Distributions

(a) 25 centres with 100 associated discs (Experiment 2)

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t</i> .	<i>P</i> .	χ^2 .	<i>n</i> .	<i>P</i> .
10 cm.	0.35	0.35	2.3304	9.36	< 0.001	—	—	—
15 cm.	0.76	0.34	3.5120	17.68	< 0.001	25.79	1	< 0.001
20 cm.	1.62	0.40	4.0002	21.11	< 0.001	94.78	3	< 0.001
25 cm.	2.64	0.42	3.4475	17.22	< 0.001	186.91	5	< 0.001
30 cm.	2.97	0.33	3.2681	15.96	< 0.001	170.15	5	< 0.001
35 cm.	4.55	0.37	4.6459	25.66	< 0.001	148.91	6	< 0.001
40 cm.	5.78	0.36	3.8118	19.79	< 0.001	92.92	7	< 0.001
Grid	0.24	0.24	2.4571	23.29	< 0.001	—	—	—

Analysis of variance of grid

		S.S.	<i>n</i> .	M.S.	<i>F</i> .	<i>P</i> .
Blocks of 256 grid units	.	0.70508	1	0.7051	1.47	> 0.2
" 128	"	3.16016	2	1.5801	3.74	0.01-0.05
" 64	"	3.66406	4	0.9160	2.17	> 0.05
" 32	"	15.54687	8	1.9434	4.61	< 0.001
" 16	"	17.09375	16	1.0683	2.53	0.001-0.01
" 8	"	28.1875	32	0.8809	2.09	< 0.001
" 4	"	53.875	64	0.8418	2.00	< 0.001
" 2	"	76.25	128	0.5957	1.41	0.01-0.05
" 1	"	108	256	0.4219	—	—

Corrected relative variance: 1.7283, *t* 8.24, *P* < 0.001

¹ It would be desirable to take such quadrats into account, but it was not considered worthwhile to try and do so at this stage.

(b) 50 centres with 75 associated discs (Experiment 3)

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t</i> .	<i>P</i> .	χ^2 .	<i>n</i> .	<i>P</i> .
10 cm.	0.40	0.40	1.5657	3.98	< 0.001	5.58	1	0.01-0.02
15 cm.	0.68	0.30	1.9275	6.53	< 0.001	13.48	1	< 0.001
20 cm.	1.28	0.32	2.2266	8.63	< 0.001	25.42	2	< 0.001
25 cm.	1.99	0.32	2.2587	8.86	< 0.001	72.78	4	< 0.001
30 cm.	2.98	0.33	1.5726	4.03	< 0.001	45.36	5	< 0.001
35 cm.	3.42	0.28	1.8559	6.02	< 0.001	27.54	5	< 0.001
40 cm.	5.03	0.31	1.5923	4.17	< 0.001	16.29	6	0.01-0.02
Grid	0.24	0.24	1.3801	6.08	< 0.001	28.89	1	< 0.001

Analysis of variance of grid

			S.S.	<i>n</i> .	M.S.	<i>F</i> .	<i>P</i> .
Blocks of 256 grid units	.	.	0.33008	1	0.3301	1.25	> 0.2
" 128	"	.	0.56641	2	0.2832	1.07	> 0.2
" 64	"	.	0.44531	4	0.1113	—	—
" 32	"	.	4.92187	8	0.6152	2.33	0.01-0.05
" 16	"	.	13.15625	16	0.8223	3.12	< 0.001
" 8	"	.	14.9375	32	0.4668	1.77	0.001-0.01
" 4	"	.	35.875	64	0.5605	2.13	< 0.001
" 2	"	.	34.75	128	0.2715	1.03	> 0.05
" 1	"	.	67.5	256	0.2637	—	—

Corrected relative variance: 1.0802, *t* 0.91, *P* 0.3-0.4

marked overdispersion. The analysis of variance of the grid results in expt. 2 shows significantly high variance for blocks of 2, 4, 8, 16, 32, and 128 units, and in expt. 3 for blocks of 4, 8, 16, and 32 units. In each case the value of the corrected relative variance is much lower than the corresponding uncorrected value, an indication of the random arrangement of individuals within the clumps. In expt. 3 it is not significantly different from unity. In expt. 2 it is significantly greater than 1, though the value obtained (1.7283) is smaller than the uncorrected value (2.4571). This may be attributed to the greater density of individuals within the clumps; a smaller grid unit would probably have shown a value still nearer unity.

These results will be more conveniently discussed after consideration of those described in the next section.

Mosaic of similarly shaped areas (Experiment 6)

Table III shows the results obtained in expt. 6. For total discs the figures for all quadrat sizes except 10 cm. indicate significant overdispersion but those for grid units do not do so. Analysis of variance of the grid data shows significantly high variance for blocks of 16, 32, and 128 units. The counts of medium density (yellow) and high density (blue) discs considered independently both show essentially similar results. The former show overdispersion for grid units and for all quadrat sizes except 10 cm., the latter for grid units and for all quadrat sizes. In the analysis of variance of grid data significantly high variance is indicated for blocks of 4, 8, 16, and 32 units for

TABLE III
Mosaic of Similarly Shaped Areas (Experiment 6)

(a) Total discs

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t.</i>	<i>P.</i>	χ^2 .	<i>n.</i>	<i>P.</i>
10 cm.	0.49	0.49	1.0099	0.07	> 0.9	0.09	1	0.7-0.8
15 cm.	1.09	0.48	1.8366	5.89	< 0.001	6.09	2	0.02-0.05
20 cm.	2.17	0.54	1.5273	3.71	< 0.001	14.72	4	0.01-0.001
25 cm.	3.48	0.56	1.8721	6.14	< 0.001	47.04	5	< 0.001
30 cm.	4.25	0.47	1.7938	5.59	< 0.001	22.52	6	< 0.001
35 cm.	5.76	0.47	1.9926	6.99	< 0.001	59.75	7	< 0.001
40 cm.	7.38	0.46	2.1373	8.00	< 0.001	61.83	8	< 0.001
Grid	0.43	0.43	0.9956	0.07	> 0.9	0.07	2	0.95-0.98

Analysis of variance of grid

		S.S.	<i>n.</i>	M.S.	<i>F.</i>	<i>P.</i>
Blocks of 256 grid units	.	0.01758	1	0.0176	—	—
" 128	"	2.72266	2	1.3613	3.47	0.01-0.05
" 64	"	2.28906	4	0.5723	1.46	> 0.05
" 32	"	8.35937	8	1.0449	2.66	0.001-0.01
" 16	"	16.15625	16	1.0098	2.57	< 0.001
" 8	"	14.9375	32	0.4668	1.19	> 0.05
" 4	"	25.875	64	0.4043	1.03	> 0.05
" 2	"	48.75	128	0.3809	—	—
" 1	"	100.5	256	0.3926	—	—

Corrected relative variance: 0.9095, *t* 1.02, *P* 0.3-0.4

(b) Blue (high density) discs alone

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t.</i>	<i>P.</i>	χ^2 .	<i>n.</i>	<i>P.</i>
10 cm.	0.34	0.34	1.3203	2.25	0.02-0.05	—	—	—
15 cm.	0.75	0.33	1.7609	5.35	< 0.001	16.49	1	< 0.001
20 cm.	1.41	0.35	2.6090	11.32	< 0.001	78.37	3	< 0.001
25 cm.	2.60	0.42	3.2789	16.04	< 0.001	237.56	4	< 0.001
30 cm.	2.42	0.27	3.8249	19.88	< 0.001	175.18	4	< 0.001
35 cm.	3.77	0.31	4.0075	21.16	< 0.001	144.37	5	< 0.001
40 cm.	4.69	0.29	4.4785	24.48	< 0.001	244.75	7	< 0.001
Grid	0.28	0.28	1.2376	3.80	< 0.001	13.25	1	< 0.001

Analysis of variance of grid

		S.S.	<i>n.</i>	M.S.	<i>F.</i>	<i>P.</i>
Blocks of 256 grid units	.	0.01758	1	0.0176	—	—
" 128	"	2.73828	2	1.3691	5.35	0.001-0.01
" 64	"	5.27344	4	1.3184	5.15	< 0.001
" 32	"	10.67187	8	1.3340	5.24	< 0.001
" 16	"	22.53125	16	1.4082	5.50	< 0.001
" 8	"	14.5625	32	0.4551	1.78	0.001-0.01
" 4	"	20.125	64	0.3145	1.23	> 0.05
" 2	"	32.75	128	0.2559	—	—
" 1	"	65.5	256	0.2559	—	—

Corrected relative variance: 0.9291, *t* 0.80, *P* 0.4-0.5

(c) *Yellow (medium density) discs alone*

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t.</i>	<i>P.</i>	χ^2 .	<i>n.</i>	<i>P.</i>
10 cm.	0.11	0.11	0.8990	0.71	0.4-0.5	—	—	—
15 cm.	0.25	0.11	1.7273	5.12	< 0.001	—	—	—
20 cm.	0.58	0.14	2.2353	8.69	< 0.001	25.83	1	< 0.001
25 cm.	0.59	0.09	1.6126	4.31	< 0.001	21.48	1	< 0.001
30 cm.	1.38	0.15	2.2073	8.50	< 0.001	80.98	3	< 0.001
35 cm.	1.54	0.13	2.4848	10.45	< 0.001	46.50	3	< 0.001
40 cm.	1.89	0.12	2.2008	8.45	< 0.001	31.53	3	< 0.001
Grid	0.12	0.12	1.1585	2.53	0.01-0.02	—	—	—

Analysis of variance of grid

			S.S.	<i>n.</i>	M.S.	<i>F.</i>	<i>P.</i>
Blocks of 256 grid units	.	.	0.00781	1	0.0078	—	—
„ 128	„	.	0.32031	2	0.1602	1.46	> 0.2
„ 64	„	.	0.82812	4	0.2070	1.89	0.05-0.2
„ 32	„	.	3.84375	8	0.4805	4.39	< 0.001
„ 16	„	.	5.75	16	0.3594	3.29	< 0.001
„ 8	„	.	6	32	0.1875	1.71	0.01-0.05
„ 4	„	.	11.75	64	0.1836	1.88	< 0.001
„ 2	„	.	17.5	128	0.1367	1.25	> 0.05
„ 1	„	.	28	256	0.1094	—	—

Corrected relative variance: 0.875, *t* 1.41, *P* 0.1-0.2

(d) *Green (low density) discs alone*

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t.</i>	<i>P.</i>	χ^2 .	<i>n.</i>	<i>P.</i>
10 cm.	0.04	0.04	0.9697	0.21	0.8-0.9	—	—	—
15 cm.	0.09	0.04	0.9192	0.57	0.5-0.6	—	—	—
20 cm.	0.18	0.04	0.8283	1.21	0.2-0.3	—	—	—
25 cm.	0.29	0.05	1.0655	0.46	0.6-0.7	—	—	—
30 cm.	0.45	0.05	1.0045	0.03	> 0.9	1.33	1	0.2-0.3
35 cm.	0.45	0.04	1.0045	0.03	> 0.9	1.33	1	0.2-0.3
40 cm.	0.80	0.05	1.2879	2.03	0.02-0.05	8.60	1	0.001-0.01
Grid	0.03	0.03	0.9706	0.47	0.6-0.7	—	—	—

Analysis of variance of grid

			S.S.	<i>n.</i>	M.S.	<i>F.</i>	<i>P.</i>
Blocks of 256 grid units	.	.	0.00781	1	0.0078	—	—
„ 128	„	.	0.03906	2	0.0195	—	—
„ 64	„	.	0.04687	4	0.0117	—	—
„ 32	„	.	0.04062	8	0.0051	—	—
„ 16	„	.	0.625	16	0.0391	1.25	> 0.05
„ 8	„	.	1.125	32	0.0352	1.12	> 0.05
„ 4	„	.	1.75	64	0.0273	—	—
„ 2	„	.	3.5	128	0.0273	—	—
„ 1	„	.	8	256	0.0312	—	—

Corrected relative variance: 1.0000, *t* 0 *P*

yellow discs and for blocks of 8, 16, 32, 64, and 128 units for blue discs. The lowest density (green) discs give no indication of non-randomness by any of the tests used except for 40-cm. quadrats. It would seem that the method is not sensitive to non-randomness in very sparsely distributed species (here 0.03125 individuals per grid unit).

The pattern formed by any one colour of discs alone is in many respects similar to that of the clumped arrangements of expts. 2 and 3, i.e. areas similar in shape and size having randomly arranged individuals, separated by areas in which the species is completely lacking, and it is worthwhile to com-

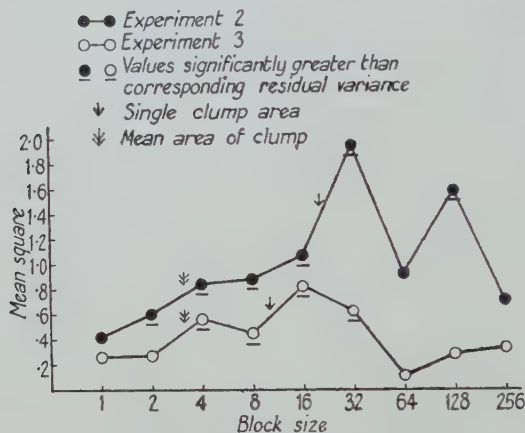


FIG. 3. Variance plotted against block size for grid data of expts. 2 and 3 (total discs).

pare the results obtained. In natural vegetation such a pattern would be likely to have causes different from those of the clumped arrangements, possibly being due to phases in a cyclic change, such as have been postulated above.

The results obtained by use of random quadrats agree in showing pronounced overdispersion if a quadrat of a certain minimum size is used. As far as they go they suggest, as might be expected, that the larger the clump size and the less dense the individuals within the clump, the larger is the minimum size of quadrat to show overdispersion and the smaller the relative variance for any one quadrat size.

The interpretation of the grid data is not so obvious. In general significantly high variance is found for certain ranges of block size, the actual range differing for the different sets of data. The range in variance with block size for any particular set of data is more easily appreciated as a graph. Figs. 3 and 4 show variance plotted against block size for expts. 2 and 3, and for the total discs, blue discs, and yellow discs of expt. 6 respectively. In each case values which are significantly greater than the residual variance within the smallest block size are indicated. It will be seen that there is a sharp rise in each graph, culminating at blocks of 32 units in expt. 2, 16 units in expt. 3, and 16 units

for blue discs and 32 units for yellow discs in expt. 6. The graph for total discs in expt. 6 will be considered later.

The nature of the clumps for any given mean density of individuals may be described in terms of such variables as density of clumps, density of individuals within clumps, mean area of clumps, number of individuals per clump, &c. These are not, of course, independent of one another and two variables may be selected which summarize the information. The most useful in vegetation analysis are (1) mean density of clumps, and (2) mean area of clump. For the present purpose it is more convenient to express the former as its reciprocal,

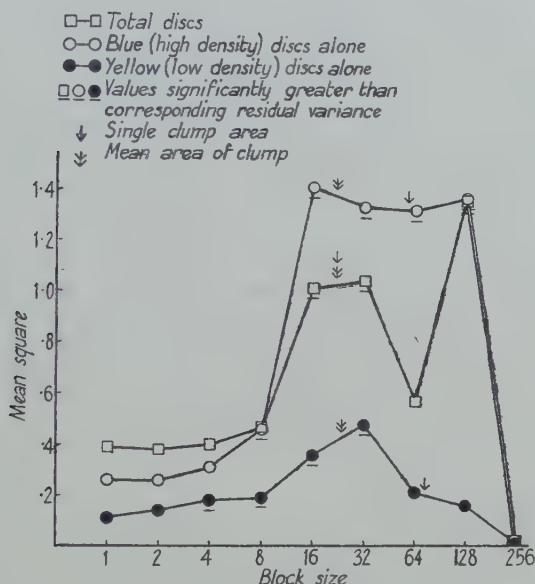


FIG. 4. Variance plotted against block size for grid data of expt. 6.

the area containing on the average one clump. This may be termed the *single clump area*, contrasting with (2) the *mean area of clump*. These values have been calculated and inserted on the graphs.

It will be seen that in expts. 2 and 3 the rise in the graph culminates with the point for the block size following the *single clump area*. In expt. 6 the peak of the curve appears to be connected rather with the *mean area of clump*, occurring at the block size before the latter for blue discs and the block size after it for yellow discs, and bearing little relation to the *single clump area*. There is thus a difference in behaviour of variance in relation to *single clump area* and *mean area of clump* in expts. 2 and 3 on the one hand and different coloured discs of expt. 6, considered separately, on the other. These layouts differ in three respects: (a) mean density of individuals, (b) proportion of the total area which is occupied by clumps, and (c) the position of clumps relative

to one another. The values of the first two factors for the experiments in question are:

	Mean density of individuals.	Area occupied by clumps.
Expt. 2	0.2441 per grid unit	78.55 grid units
Expt. 3	0.2441 "	157.10 "
Expt. 6 (yellow discs) . .	0.1250 "	166.80 "
Expt. 6 (blue discs) . .	0.2754 "	202.80 "

The difference in behaviour cannot be correlated with difference in mean density of individuals. Consideration of the area occupied by clumps shows that the two groups of experiments do not overlap but the difference within the groups is considerably greater than the difference between expt. 3 and expt. 6 (yellow discs) and the differing behaviour can hardly be attributed to this factor. It should be noted that the values for expts. 2 and 3 are somewhat exaggerated as in these layouts it was possible for clumps to overlap, but the correction is small and not sufficient to affect materially this conclusion.

We are left then with the third factor, the position of the clumps relative to one another. In expts. 2 and 3 the clumps are themselves arranged randomly (see data above for centre discs alone). In expt. 6, however, they are more or less regularly arranged (see Fig. 2), i.e. they are underdispersed. This appears to be the fundamental difference between the groups. It might be suggested then from empirical consideration that where a species is distributed in a clumped manner the analysis of grid data will show variance rising sharply with block size and culminating in a peak, the peak corresponding approximately to the *single clump area* if the clumps are randomly distributed and to the *mean area of clump* if they are underdispersed. This rise in variance with increasing block size will of course occur only if the grid unit is smaller than the *mean area of clump*.

It must be emphasized that this approach is only an empirical one and it is clearly desirable that some consideration of theoretical expectation be made. Professor Bartlett (personal communication) has made a preliminary examination of the problem and his provisional conclusions may be briefly summarized as follows: (1) Variance may be expected to rise to a maximum value as block size reaches the *mean area of clump*, diminishing thereafter if the clumps are underdispersed. (2) If the clumps are randomly distributed the maximum variance will again tend to be reached at the *mean area of clump* but will be maintained at about the same value with increasing block size. There is no apparent reason why the *single clump area* should be critical in determining the position of the peak though it will have an effect on its magnitude. (3) If the clumps are themselves overdispersed the variance will also be maintained. The interpretation made above of the variance graph for expts. 2 and 3 is thus suspect; the peaks obtained in the graphs could in fact be considered due to chance fluctuation, the degrees of freedom on which the variances for larger block sizes are based being small (pp. 297-8 above).

The object of these experiments is, of course, to enable conclusions on the nature of the distributions of individuals to be drawn from field data. If a

peaked variance graph is found from field data, how is it to be interpreted? The peak may be an indication of *mean area of clump* or it may, as expts. 2 and 3 have shown, result from chance. If clumps are randomly distributed or overdispersed, yet distinct from one another, they must be comparatively sparse. In such a case the pattern will generally be apparent on inspection in the field. If inspection fails to reveal clumping the clumps must be comparatively large relative to the distances between them, in which case, if they are distinct from one another, they will necessarily be more or less underdispersed. It may therefore generally be assumed that where clumping is not revealed on inspection the peak of the variance graph corresponds to the *mean area of clump*.

It remains to consider the data for total discs of expt. 6. When the whole area is occupied by a species but at different densities in different parts the pattern is comparable to one of clumps where the clumps have become contiguous. (In this case the *mean area of clump* and *single clump area* will have the same value.) It will be seen from Fig. 4 that the graph shows a sharp rise culminating at about this point, although a higher value is reached at a larger block size after intermediate fluctuations. As mentioned above, such fluctuations at the largest block sizes are not unexpected. It seems therefore that the same interpretation may be put on data from such a pattern as on that from underdispersed and distinct clumps. This conclusion is the more important in that it applies to the pattern the detection of which is likely to be the most difficult by subjective methods.

Mosaic of irregularly shaped areas (Experiments 4 and 5)

The results of expts. 4 and 5 are given in Tables IV and V respectively. For yellow or green discs alone, in both experiments, the relative variance test indicates significant overdispersion for the larger quadrat sizes, for 30-cm. and larger quadrats in expt. 5 (green discs), 20-cm. and larger in expt. 4 (green discs), 25-cm. and larger in expt. 4 (yellow discs), and 15-cm. and larger in expt. 5 (yellow discs). These results are not unexpected. In each case overdispersion is indicated for all quadrat sizes larger than a certain

TABLE IV

Mosaic of Irregularly Shaped Areas (Experiment 4)

(a) *Total discs*

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	<i>t.</i>	<i>P.</i>	χ^2 .	<i>n.</i>	<i>P.</i>
10 cm.	0.26	0.26	1.0583	0.41	0.6-0.7	—	—	—
15 cm.	0.57	0.25	0.8242	1.24	0.1-0.2	0.37	1	0.5-0.7
20 cm.	1.06	0.26	0.6636	2.37	0.01-0.02	9.47	2	0.001-0.01
25 cm.	1.62	0.26	0.7954	1.44	0.1-0.2	5.47	3	0.1-0.2
30 cm.	2.34	0.26	0.7962	1.43	0.1-0.2	11.15	4	0.02-0.05
35 cm.	2.99	0.24	0.7263	1.93	0.05-0.1	7.74	5	0.1-0.2
40 cm.	4.21	0.26	0.6588	2.40	0.01-0.02	8.86	6	0.1-0.2
Grid	0.21	0.21	0.9306	1.11	0.2-0.3	0.89	1	0.3-0.5

Analysis of variance of grid

			S.S.	n.	M.S.	F.	P.
Blocks of 256 grid units	.	.	0.03225	1	0.0322	—	—
" 128 "	.	.	0.13281	2	0.0664	—	—
" 64 "	.	.	0.29687	4	0.0742	—	—
" 32 "	.	.	1.21875	8	0.1523	—	—
" 16 "	.	.	2.5625	16	0.1602	—	—
" 8 "	.	.	9.625	32	0.3008	1.60	0.01-0.05
" 4 "	.	.	16	64	0.25	1.33	> 0.05
" 2 "	.	.	24.5	128	0.1914	1.02	> 0.05
" 1 "	.	.	48	256	0.1875	—	—

Corrected relative variance: 0.8727, t 1.44, P 0.1-0.2(b) *Yellow (high density) discs alone*

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	t .	P .	χ^2 .	n .	P .
10 cm.	0.13	0.13	1.1896	1.33	0.1-0.2	—	—	—
15 cm.	0.29	0.13	1.2048	1.44	0.1-0.2	—	—	—
20 cm.	0.58	0.14	1.0164	0.12	> 0.9	0.41	1	0.5-0.7
25 cm.	0.93	0.15	1.3523	2.48	0.01-0.02	19.97	2	< 0.001
30 cm.	1.29	0.14	1.6803	4.79	< 0.001	26.91	2	< 0.001
35 cm.	1.33	0.11	1.7324	5.15	< 0.001	23.86	2	< 0.001
40 cm.	2.23	0.14	2.0732	7.55	< 0.001	88.53	4	< 0.001
Grid	0.11	0.11	1.0612	0.98	0.3-0.4	—	—	—

Analysis of variance of grid

			S.S.	n.	M.S.	F.	P.
Blocks of 256 grid units	.	.	0.19531	1	0.1953	2.17	0.1-0.2
" 128 "	.	.	0.20312	2	0.1016	1.13	> 0.05
" 64 "	.	.	1.59375	4	0.3984	4.43	0.01-0.05
" 32 "	.	.	2.625	8	0.3281	3.65	< 0.001
" 16 "	.	.	2.5625	16	0.1602	1.78	0.01-0.05
" 8 "	.	.	4	32	0.125	1.39	> 0.5
" 4 "	.	.	10.75	64	0.1680	1.87	< 0.001
" 2 "	.	.	16.5	128	0.1289	1.43	0.001-0.01
" 1 "	.	.	23	256	0.0898	—	—

Corrected relative variance: 0.7931, t 2.34, P 0.01-0.02(c) *Green (low density) discs alone*

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	t .	P .	χ^2 .	n .	P .
10 cm.	0.13	0.13	1.1896	1.33	0.1-0.2	—	—	—
15 cm.	0.28	0.12	0.9437	0.40	0.6-0.7	—	—	—
20 cm.	0.48	0.12	1.1566	1.10	0.2-0.3	5.38	1	0.02-0.05
25 cm.	0.69	0.11	1.3671	2.58	0.01-0.02	7.54	1	0.01-0.02
30 cm.	1.05	0.12	1.5464	3.85	< 0.001	8.61	2	0.01-0.02
35 cm.	1.66	0.14	1.7673	5.40	< 0.001	50.24	3	< 0.001
40 cm.	1.98	0.12	1.8873	6.24	< 0.001	42.39	4	< 0.001
Grid	0.10	0.10	0.9773	0.36	0.7-0.8	—	—	—

Analysis of variance of grid

				S.S.	n.	M.S.	F.	P.
Blocks of 256 grid units	.	.	.	0.07031	1	0.0703	—	—
" 128	"	.	.	0.66406	2	0.3320	3.54	0.01-0.05
" 64	"	.	.	1.89062	4	0.4727	5.04	< 0.001
" 32	"	.	.	1.21875	8	0.1523	1.62	> 0.05
" 16	"	.	.	1.125	16	0.0731	—	—
" 8	"	.	.	3	32	0.0937	—	—
" 4	"	.	.	8.25	64	0.1289	1.37	0.01-0.05
" 2	"	.	.	10.5	128	0.0820	—	—
" 1	"	.	.	24	256	0.0937	—	—

Corrected relative variance: 0.9225, t 0.88, P 0.3-0.4

TABLE V

Mosaic of Irregularly Shaped Areas (Experiment 5)

(a) *Total discs*

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	t .	P .	χ^2 .	n .	P .
10 cm.	0.27	0.27	0.8122	1.32	0.1-0.2	—	—	—
15 cm.	0.58	0.26	1.0164	0.12	> 0.9	0.03	1	0.8-0.9
20 cm.	1.13	0.28	1.0665	0.47	0.6-0.7	2.36	2	0.3-0.5
25 cm.	1.96	0.31	1.6483	4.56	< 0.001	5.59	3	0.1-0.2
30 cm.	2.65	0.29	1.1464	1.03	0.3-0.4	5.54	5	0.3-0.5
35 cm.	3.55	0.29	1.2655	1.87	0.05-0.1	7.86	5	0.1-0.2
40 cm.	4.52	0.28	1.6603	4.65	< 0.001	11.82	6	0.05-0.1
Grid	0.24	0.24	0.9855	0.23	0.8-0.9	0.13	1	0.7-0.8

Analysis of variance of grid

				S.S.	n.	M.S.	F.	P.
Blocks of 256 grid units	.	.	.	0.03125	1	0.0312	—	—
" 128	"	.	.	0.01562	2	0.0078	—	—
" 64	"	.	.	3.26562	4	0.8164	3.54	0.01-0.05
" 32	"	.	.	2.09375	8	0.2617	1.14	> 0.05
" 16	"	.	.	4.4375	16	0.2773	1.20	> 0.05
" 8	"	.	.	9.875	32	0.3086	1.34	> 0.05
" 4	"	.	.	16.75	64	0.2617	1.14	> 0.05
" 2	"	.	.	26.5	128	0.2070	—	—
" 1	"	.	.	59	256	0.2305	—	—

Corrected relative variance: 0.9516, t 0.55, P 0.5-0.6

(b) *Yellow (high density) discs alone*

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	t .	P .	χ^2 .	n .	P .
10 cm.	0.20	0.20	0.9091	0.64	0.5-0.6	—	—	—
15 cm.	0.40	0.18	1.3636	2.56	0.01-0.02	3.91	1	0.02-0.05
20 cm.	0.84	0.21	1.6527	4.59	< 0.001	14.16	2	< 0.001
25 cm.	1.57	0.25	2.4738	10.37	< 0.001	62.15	3	< 0.001
30 cm.	1.97	0.22	2.3223	9.31	< 0.001	89.03	3	< 0.001
35 cm.	2.76	0.23	2.2553	8.83	< 0.001	107.72	5	< 0.001
40 cm.	3.55	0.22	2.9670	13.84	< 0.001	85.08	5	< 0.001
Grid	0.19	0.19	1.1114	1.78	0.05-0.1	6.80	1	0.001-0.01

Analysis of variance of grid

			S.S.	n.	M.S.	F.	P.
Blocks of 256 grid units	.	.	0.04883	1	0.0488	—	—
" 128	"	.	0.05078	2	0.0254	—	—
" 64	"	.	6.91406	4	1.7285	7.31	< 0.001
" 32	"	.	2.95312	8	0.3691	1.56	> 0.05
" 16	"	.	5.09375	16	0.3184	1.35	> 0.05
" 8	"	.	10.4375	32	0.3262	1.38	> 0.05
" 4	"	.	13.625	64	0.2129	—	—
" 2	"	.	5.75	128	0.0449	—	—
" 1	"	.	60.5	256	0.2363	—	—

Corrected relative variance: 1.2737, t 3.10, P 0.001–0.01

(c) Green (low density) discs alone

Quadrat size.	Mean.	Mean per 100 cm. ²	Relative variance.	t .	P .	χ^2 .	n .	P .
10 cm.	0.07	0.07	0.9394	0.43	0.6–0.7	—	—	—
15 cm.	0.18	0.08	1.0527	0.37	0.7–0.8	—	—	—
20 cm.	0.29	0.07	0.9958	0.03	> 0.9	—	—	—
25 cm.	0.39	0.06	1.1342	0.94	0.3–0.4	1.56	1	0.2–0.3
30 cm.	0.68	0.08	1.3928	2.76	0.001–0.01	4.00	1	0.02–0.05
35 cm.	0.79	0.06	1.5674	3.99	< 0.001	2.29	1	0.1–0.2
40 cm.	0.97	0.06	1.6340	4.46	< 0.001	26.14	2	< 0.001
Grid	0.06	0.06	0.9452	0.88	0.3–0.4	—	—	—

Analysis of variance of grid

			S.S.	n.	M.S.	F.	P.
Blocks of 256 grid units	.	.	0.00195	1	0.0020	—	—
" 128	"	.	0.01953	2	0.0098	—	—
" 64	"	.	0.78906	4	0.1973	3.74	0.01–0.05
" 32	"	.	0.64062	8	0.0801	1.52	> 0.05
" 16	"	.	0.21875	16	0.0137	—	—
" 8	"	.	1.5625	32	0.0488	—	—
" 4	"	.	3.875	64	0.0605	1.15	> 0.05
" 2	"	.	6.75	128	0.0527	—	—
" 1	"	.	13.5	256	0.0527	—	—

Corrected relative variance: 0.9310, t 0.78, P 0.4–0.5

value, the minimum value decreasing with increasing density of individuals (the order in which the experiments are enumerated above). The minimum quadrat size for which non-randomness is indicated in each case confirms the conclusion already reached that a quadrat size giving a mean approaching one individual per quadrat will provide a more sensitive test of randomness than the smaller sizes that have generally been used.

The data for total discs do not give any such regular indication of non-randomness. In expt. 5 overdispersion is indicated by the relative variance test for 25- and 40-cm. quadrats and in expt. 4 underdispersion is indicated for 20- and 40-cm. quadrats. When these four sets of data are compared with the corresponding Poisson expectations only one (20-cm. quadrats of expt. 5)

shows a significantly high value of χ^2 .¹ This is an unexpected and unusual discrepancy, although the reverse case of no significant over- or underdispersion, but significant departure from Poisson expectation is not unusual in the field, as Blackman (1942) has shown. Such a result can clearly only occur when the deviation for any individual entry in the table is small, particularly when the expected value itself is small. Professor E. Ashby (personal communication) has met with this behaviour occasionally in the field, but it has not usually been considered necessary to apply the χ^2 test where the relative

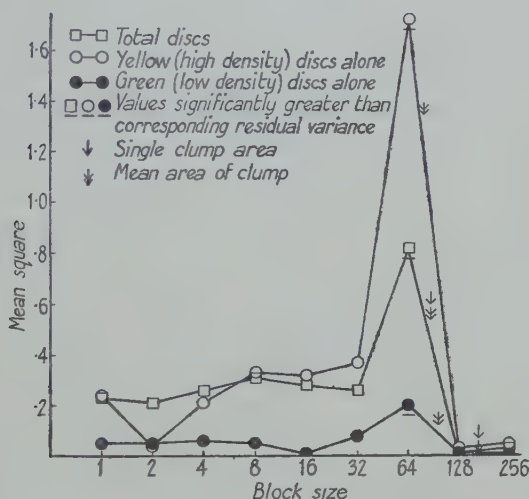


FIG. 5. Variance plotted against block size for grid data of expt. 5.

variance shows significant deviation from unity, and it may in fact be commoner than appears. The data are not sufficient to suggest that this type of discrepancy is characteristic of the type of layout considered, but its occurrence in both experiments is interesting.

The occurrence of underdispersion in expt. 4 calls for comment. Although the data for three quadrat sizes only show significant underdispersion by either test, those for all sizes except 10 cm. gave a relative variance of less than 1. Bearing in mind that the difference in density of the two colours is comparatively small, it seems safest at the present stage to attribute the underdispersion to faults in the randomizing of the layout. The total disc data of expt. 4 show other anomalous features (see below).

It is more convenient to consider the grid data for expt. 5 before that for expt. 4. For total discs and for green and yellow discs considered separately a significantly high variance is indicated only for blocks of 64 grid units. When variance is plotted against block size (Fig. 5) a peaked curve is obtained in each case comparable with those of expt. 6, with the peak, as in that experi-

¹ The data for 30-cm. quadrats in expt. 4, on the other hand, show a significantly high χ^2 although no significant underdispersion is indicated by the relative variance test.

ment, corresponding to the value of the *mean area of clump*. Thus even with the more irregular layout used in this experiment the variance behaves in a similar manner to that observed in a mosaic of regularly shaped areas and an estimate of the *mean area of clump* may be obtained from the grid data.¹

The grid data for the green and yellow discs of expt. 4 confirm this result (Fig. 6). Though a wider range of block sizes shows significantly high variance there is again a pronounced peak in the graph of variance against block size, corresponding to the *mean area of clump*. The data for total discs, however, have a peak at blocks of 8 units (the variance for which is the only significantly

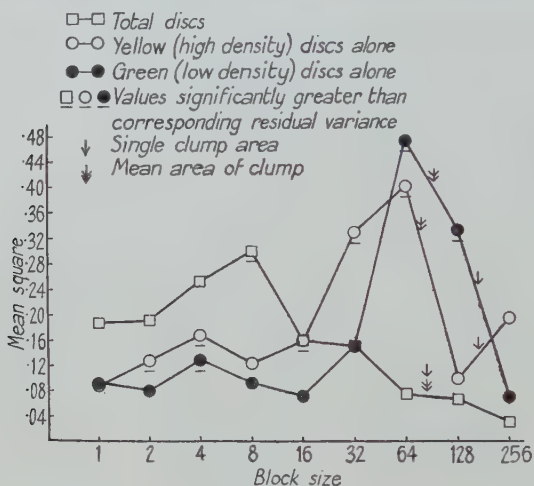


FIG. 6. Variance plotted against block size for grid data of expt. 4.

high one). This behaviour is anomalous, and it must be assumed that the method is not sensitive to the comparatively small difference, 0.0615 discs per grid unit, in density between high and low density areas. The significantly high variance for blocks of 8 units may perhaps be attributed to the fault in randomization already postulated.

CONCLUSIONS

Only a small proportion of the numerous patterns of individuals that are known or might be expected to occur in natural vegetation have been examined. The object has not been to make an extensive survey, but rather to consider whether problems of the nature of the structure of plant communities can be attacked by the simple and convenient technique of quadrat counts to give information other than the bare statement that a species is or is not randomly distributed. Before further theoretical analysis can profitably be undertaken it is necessary to try out the methods suggested on natural vegetation. This is being undertaken. In the meantime some tentative conclusions can be drawn as to the methods that can profitably be employed. It is clear

¹ In calculating *single clump area* and *mean area of clump* the large U-shaped area of low density has been considered equivalent to two clumps.

that random throws of one quadrat size are not sufficient to determine whether a species is randomly distributed. A series of throws of quadrats of varying size will test the randomness or otherwise of the distribution of a species provided that sufficiently large quadrats are taken to reach a mean of one individual per quadrat. It is necessary to test the counts obtained both by the relative variance test and against Poisson expectation by the χ^2 test. If the distribution is not random the amount of information about the type of heterogeneity obtainable by random quadrats is limited, though if a large enough series of quadrat sizes is used the trend of relative variance with quadrat size may give some information about its nature.

A grid of contiguous quadrats, on the other hand, provided that its dimensions are such that its units can be associated into blocks of increasing size, and that the size of the grid unit is small enough to have a mean of much less than one individual, can provide all the information obtainable from random quadrats. (Satisfaction of the second condition may be a difficulty in such communities as grassland, where a grid unit small enough to include a reasonably high proportion of blank units for an abundant species may approach the size of an individual, when the Poisson expectation no longer applies.) Furthermore, use of a grid will give information on the type of heterogeneity not available from random quadrats. Other disadvantages of a grid are likely to be practical ones. Time is needed to lay out the grid before enumeration can be commenced; and if a very small grid unit is needed the sampling of a large enough area to be representative may be laborious. On the other hand, use of a grid opens the way to analysis of forest and other communities where the use of random quadrats is impracticable. In general the advantages of a grid are such that its use is preferable wherever possible.

SUMMARY

The use of random quadrats in the determination of abundance is limited owing to the non-random distribution of many species.

The nature of such non-randomness is discussed, and it is suggested that it may be caused in some cases by the occurrence of a mosaic pattern of phases, the phases having the same species at different densities.

Artificial 'communities' of discs have been constructed and sampled by random throws of quadrats of different sizes and by a grid of contiguous quadrats. The variance shown by the grid data has been analysed into components contributed by blocks of grid units of increasing size.

These experiments have shown

1. The use of a single quadrat size is not sufficient to determine the randomness or otherwise of distribution of a species.
2. Grid sampling of clumped distribution, if the clumps themselves are underdispersed, gives a graph of variance against block size having a pronounced peak. This peak occurs at a block size corresponding to the *mean area of clump*.

3. If the clumps are random or overdispersed a peaked graph may be obtained, but no significance can be attached to the position of the peak. Such distributions are, however, likely to be detectable by observation in the field.
4. Grid sampling of a mosaic of phases having the same species at different densities gives a peaked variance/block size graph, with the peak corresponding to the *mean area of clump*, i.e. to the mean size of mosaic unit.

The use of a grid opens the way to more detailed study of the nature of the non-random distribution of species in the field, especially to the detection of mosaic patterns not normally revealed by subjective methods.

It gives me great pleasure to express my thanks to Professor E. Ashby and Professor M. S. Bartlett for their helpful criticism and stimulating advice, and to Mr. J. H. Darwin for determining the standard error of the corrected relative variance.

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NOTE

A note on the viability of potato seeds.—The breeding programme of the potato section of the Scottish Society for Research in Plant Breeding involves the collection, each year, of a considerable quantity of seed. It is unusual for all the seed produced in any one year to be sown during the year following, and the remainder forms a reserve of breeding material which is drawn upon as required. In this way there has accumulated a seed collection which contains samples of the seed produced each year for the past 24 years, and it was recently thought desirable to ascertain how much of this seed would in fact be of use if wanted. Accordingly, germination tests have been made, with the results given below. Nothing more recent than 9-year-old seed was used for the tests, since experience had shown that seed up to 10 years old could be relied on to give satisfactory results.

Each sample contained 200 seeds and there were two samples per year of sampling. The seed was sown in compost in shallow pans. The criterion for germination was taken as the unfolding of the cotyledons from the seed-coat, and the figure for percentage germination was calculated 21 days after the emergence of the first seedling. As will be seen from Table I, germination subsequent to this period was negligible when viability was at its maximum, but could be quite appreciable in freshly harvested seed.

The nature of the material being sampled, which was the product of a progressive breeding programme, resulted in the introduction of an unknown factor—the effect, if any, of parentage on germination capacity; for, since any particular cross was seldom repeated in more than one year, it followed that the seed samples were unavoidably of different ancestry. The breeding programme makes use, not only of the many varieties of *Solanum tuberosum*, but also of other species of *Solanum* (*S. demissum*, *S. Rybinii*, *S. simplicifolium*, &c.) of which the germination capacity may differ from that of *S. tuberosum*. This effect was reduced in any one year's tests by taking the two samples from different crosses and averaging the results, but it still remained when results from different years were compared. However, the object of the test was rather to get a trend than a collection of absolute figures, and it is suggested that this trend is quite clear.

In addition to testing samples taken from each year's collection, a more detailed test was made of seed in its first year of life. Here any effect of parentage was reduced to a minimum by using a bulk sample, drawn from many crosses, and taking from it small samples for the individual tests. Berries were picked at random from the progeny of the previous year's crosses; they were stored until they were fully ripe—the berries shrunken, the skin soft and easily broken, the pulp soft and easily separated from the seed—when the seed was extracted, dried, bulked, and sampled, and the first sample sown at once. Successive samples were sown each week for 4 weeks, then each 4 weeks for a year.

The tests show that some seeds will germinate immediately the berry is ripe, but that full germinative capacity is reached only 5–8 weeks after that time, and that this capacity is retained thereafter for about 13 years, after which it falls rapidly. These results agree generally with those of Wollenweber (1942), who got

full germination up to 13 years, with some viability even after 20 years. Minor differences may result from the effect of parentage noted above, or from differences in methods of storage; Wollenweber's seeds were stored in glass bottles at room temperature, and the seeds used in the tests now described were in seed packets stored in a wood-lined metal box, also at room temperature.

It will be seen from Table I that the interval between sowing and the appearance of the first seedling varies, though not widely, throughout the year, and it is likely that this variation is caused by fluctuations in the mean temperature of the greenhouse in which the tests were made. The sowings in the annual series were made in November and the first of the weekly series in December, and the time required for germination is distinctly shorter during the warmer months of the year.

It emerged from these tests that continued inbreeding has a deleterious effect on germination as will be seen from the comparative figures in Table II.

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TABLE I

Age of seed.	Percentage germination in 21 days.	Total percentage germination.	No. of days from sowing to appearance of	
			first seedling.	last seedling.
Fresh	45.5	75.5	14	99
1 week	49.5	69.0	15	93
2 weeks	43.0	81.5	13	86
3 "	35.0	85.5	12	100
4 "	42.5	71.5	10	94
8 "	87.0	94.5	13	58
12 "	93.5	94.5	9	37
16 "	95.5	97.0	9	37
20 "	93.5	95.0	7	30
24 "	88.0	92.0	8	40
28 "	95.0	95.0	9	23
32 "	91.0	91.0	7	21
36 "	78.5	79.0	8	44
40 "	91.0	91.5	12	48
44 "	87.5	88.0	8	40
48 "	73.5	73.5	11	27
52 "	71.5	74.5	12	46
9 years	85.5	86.5	14	41
10 "	87.5	89.5	13	51
11 "	66.5	68.5	13	54
12 "	67.5	73.0	15	61
13 "	54.0	61.5	16	61
14 "	1.5	3.5	28	55
15 "	11.5	13.5	19	55
16 "	11.5	18.0	19	61
17 "	—	—	—	—
and over				

TABLE II

Origin of seed.	Age of seed.	Percentage germination.
Hybrid	11 years	66.5
9th generation of selfing	11 "	12.5
Hybrid	12 "	67.5
8th generation of selfing	12 "	3.5
Hybrid	13 "	54.0
6th generation of selfing	13 "	—

